





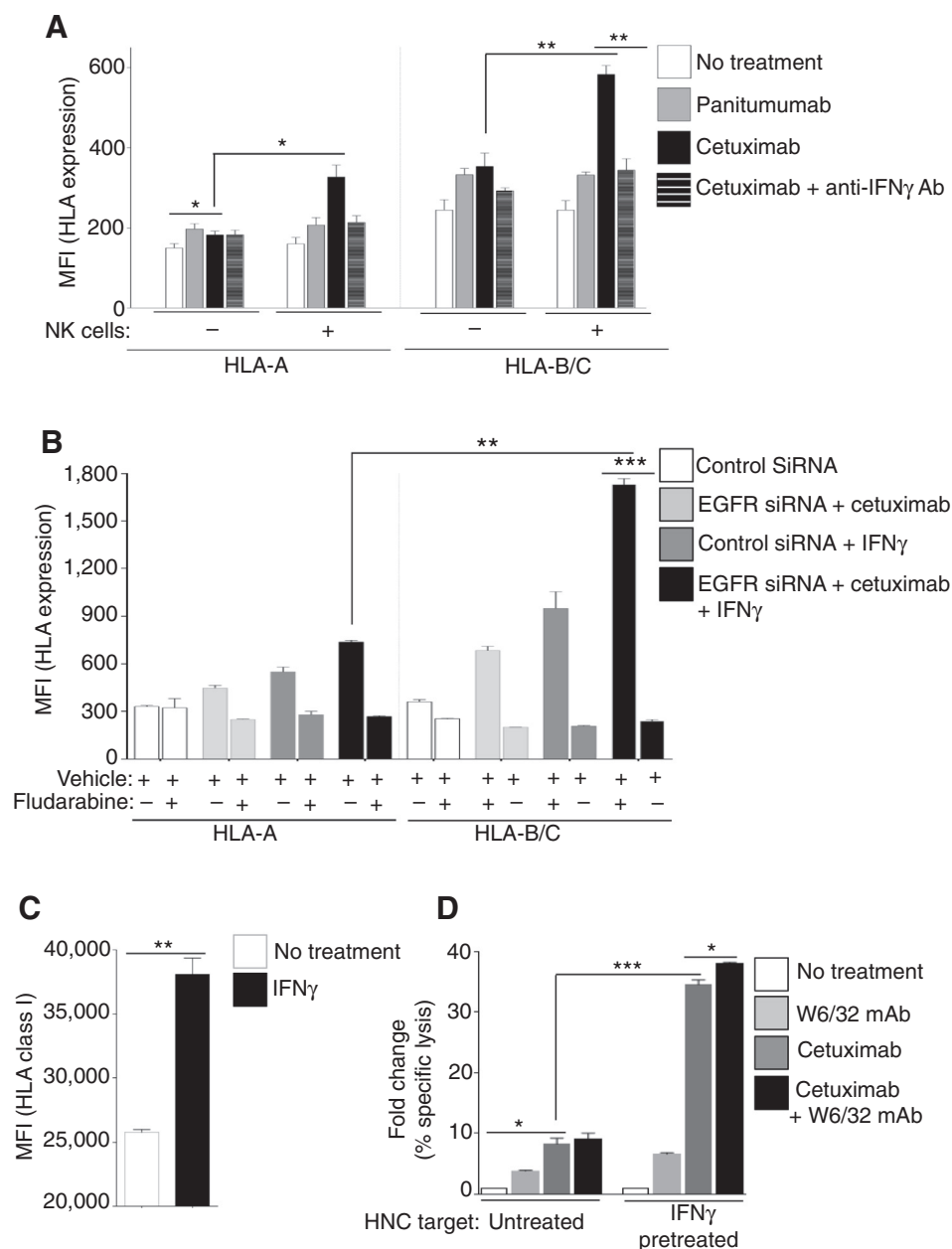






**Figure 4.**

Cetuximab-activated NK cells and IFN $\gamma$  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  $\mu$ g/mL), cetuximab (IgG1, 10  $\mu$ g/mL). Levels of HLA-A (left) or HLA-B/C (right) were determined by FACS. In parallel, polyclonal anti-IFN $\gamma$  Ab (10  $\mu$ g/mL) was added at indicated conditions to determine the effect of IFN $\gamma$  released from cetuximab-activated NK cells. B, HNC cells were pretreated with fludarabine (20  $\mu$ mol/L), and after EGFR siRNA or cetuximab treatment (48 hours, 10  $\mu$ g/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 IFN $\gamma$  (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  $^{51}$ Cr release assay, 40:1 E/T ratio) against untreated or IFN $\gamma$  pretreated HNC targets (C), were independently evaluated in presence of mAb W6/32 (50  $\mu$ g/mL), cetuximab (10  $\mu$ g/mL) or combination of mAb W6/32 plus cetuximab. The ratio of NK cell cytotoxicity against untreated HNC targets, and IFN $\gamma$ -treated HNC targets is shown. Results represent mean  $\pm$  SEM from three independent experiments; \*,  $P < 0.05$ ; \*\*,  $P < 0.001$ ; \*\*\*,  $P < 0.0001$ . MFI, mean fluorescence intensity.



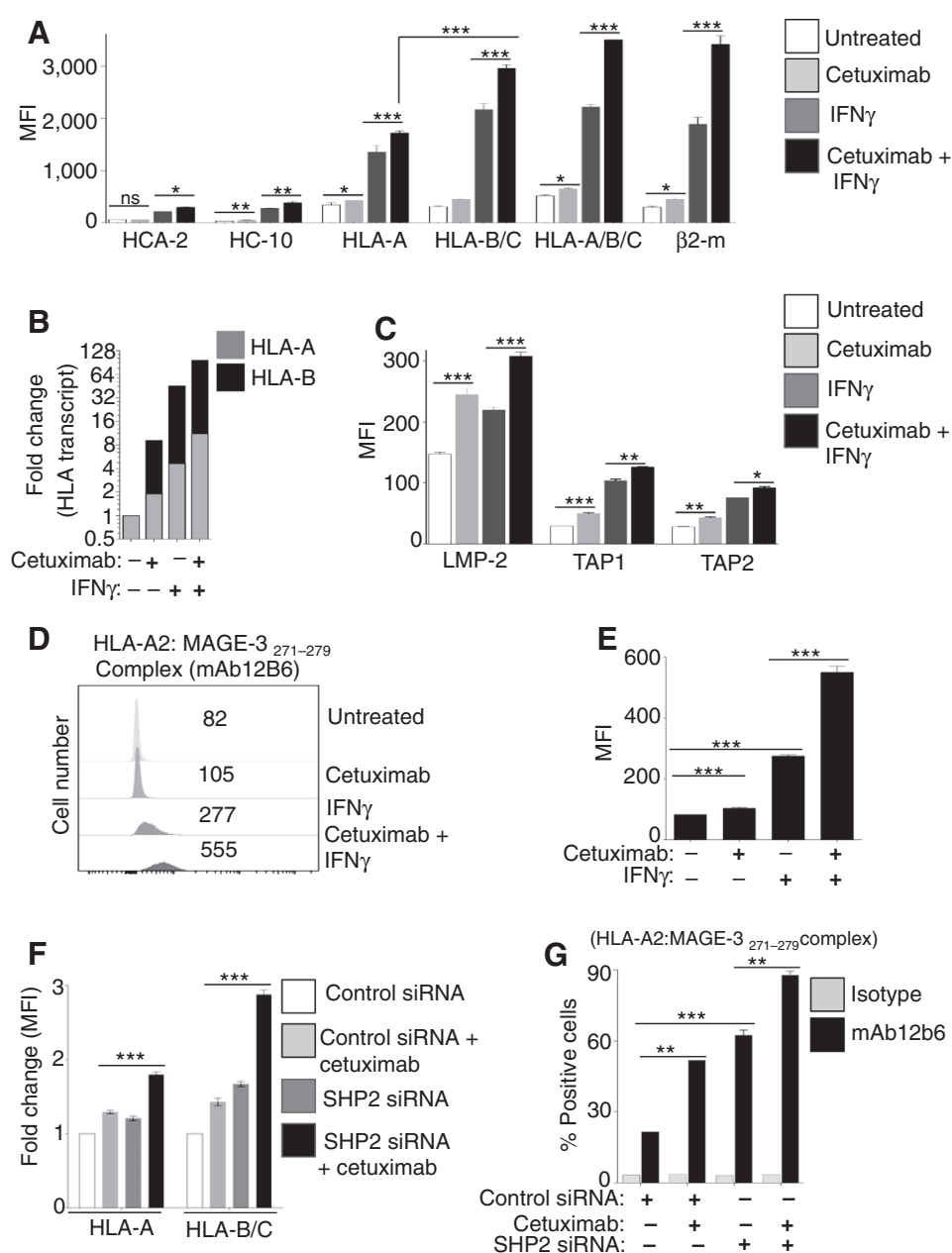
HLA-A. In support of a common pathway, the STAT1 inhibitor fludarabine (23) abrogated HLA-A and HLA-B/C upregulation in response to cetuximab, EGFR siRNA, or IFN $\gamma$  treatment (Fig. 4B). We further evaluated the contribution of IFN $\gamma$ -induced HLA class I expression to NK-cell-mediated antitumor effects (Fig. 4C). Cetuximab-mediated antibody-dependent cellular cytotoxicity (ADCC) was significantly enhanced against IFN $\gamma$ -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 IFN $\gamma$  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  $\beta$ 2-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); IFN $\gamma$  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 IFN $\gamma$  and cetuximab was used. We also found greater HLA-B versus HLA-A allele transcripts after treatment with cetuximab, with IFN $\gamma$  alone, or with cetuximab plus IFN $\gamma$  (Fig. 5B and Supplementary Fig. S3A–S3E).

Srivastava et al.

**Figure 5.**

SHP2 inhibition robustly enhances cetuximab-mediated TA presentation. A, JHU-029 cells were treated with cetuximab (10  $\mu$ g/mL, 72 hours), IFN $\gamma$  (10 U/mL, 72 hours), cetuximab plus IFN $\gamma$  (10  $\mu$ g/mL, 10 U/mL, respectively, 72 hours) and levels of intracellular-free HLA class I A or B, HLA-A, HLA-B/C, HLA-A/B/C,  $\beta$ 2-m were determined by FACS. B, under above described conditions the transcript levels of HLA-A and HLA-B were determined by qPCR. C, similarly, in the above described conditions, levels of LMP2, TAP1, and TAP2 were determined by FACS. In HLA-A2<sup>+</sup> JHU-022 cells presentation of HLA-A2:MAGE-3<sub>271-279</sub>-peptide complex (probed by mAb 12B6, followed by FITC-(Fab)<sup>2</sup> was determined with flow cytometry after treatment with cetuximab (10  $\mu$ g/mL), IFN $\gamma$  (10 U/mL), cetuximab plus IFN $\gamma$  (10  $\mu$ g/mL, 10 U/mL, respectively, 72 hours). D, representative histogram. E, graphical presentation of MFI values. F, HNC JHU-029 cells were treated with SHP2 siRNA or control siRNA. After 24 hours, cells were treated with cetuximab (10  $\mu$ g/mL, 48 hours), and levels of HLA-A and HLA-B/C were evaluated by FACS. G, levels of HLA-A2:MAGE-3<sub>271-279</sub>-peptide complex (clone mAb 12b6) presentation were determined by FACS after treatment with control siRNA, control siRNA plus cetuximab (5  $\mu$ g/mL), SHP2 siRNA, and SHP2 siRNA plus cetuximab (additional 48 hours) in MAGE-3<sub>271-279</sub> TA-positive HLA-A2<sup>+</sup> PCI-13. Results represent mean  $\pm$  SEM from three independent experiments; \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.001$ ; \*\*\*,  $P \leq 0.0001$ . MFI, mean fluorescence intensity.

Because cetuximab treatment enhanced the expression of LMP2, and TAP1/2 (Fig. 5C and Supplementary Fig. S4A–S4B), 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-3<sub>271-279</sub> complex (Supplementary Fig. S5A–S5B), to quantitatively measure levels of surface HLA-TA complexes. Cetuximab enhanced the HLA-A2:MAGE-3<sub>271-279</sub> complex ( $P < 0.001$ ; Fig. 5D and E), which was even more robust after IFN $\gamma$  treatment ( $P < 0.0001$ ). Interestingly, the combination of cetuximab and IFN $\gamma$  treatment evoked the highest level of HLA-A2:MAGE-3<sub>271-279</sub>-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

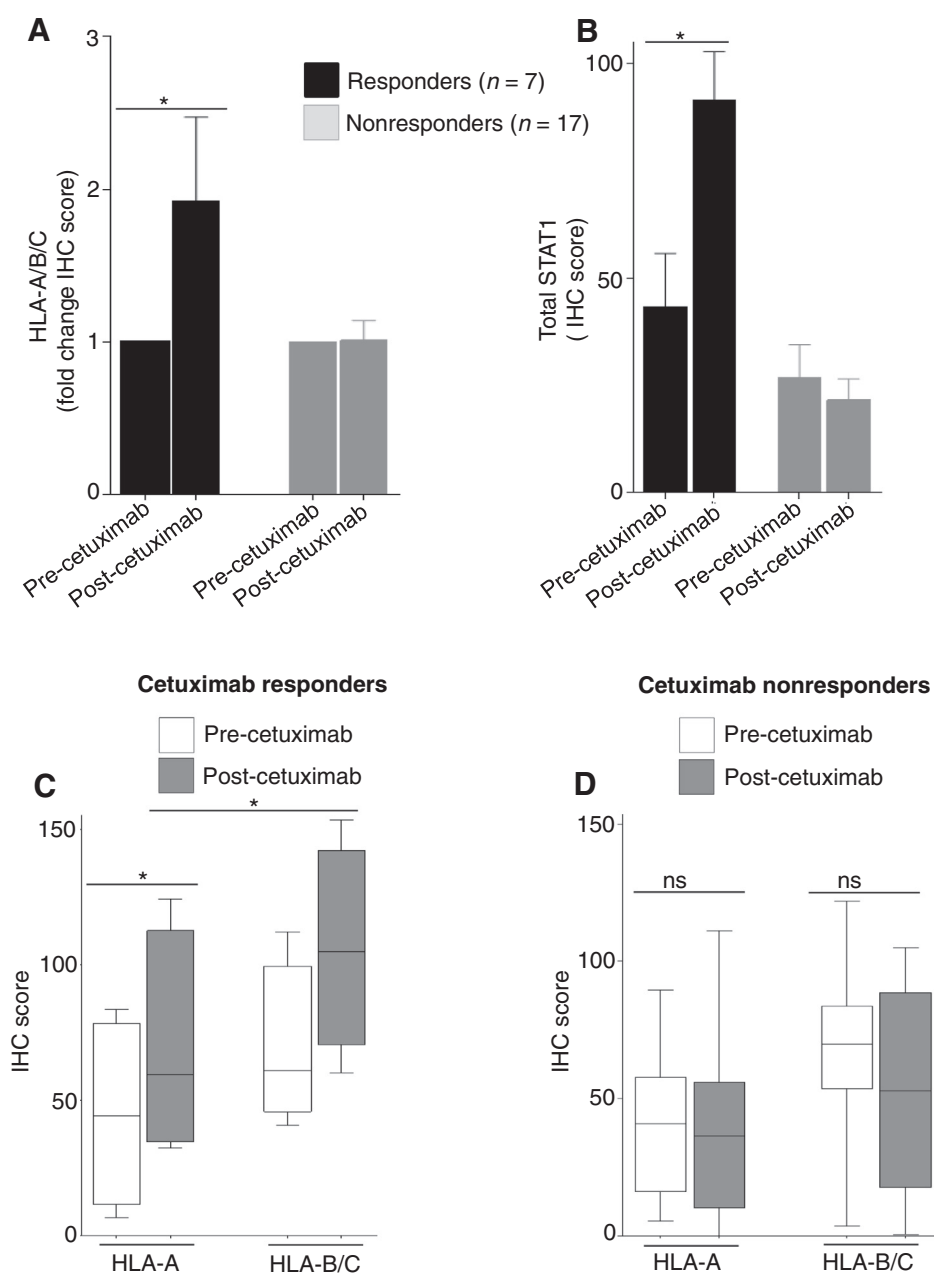
alleles (Fig. 5F and Supplementary Fig. S5C). SHP2 depletion in HLA-A2<sup>+</sup> HNC cells also enhanced HLA-A2:MAGE-3<sub>271-279</sub>-peptide presentation after cetuximab treatment (Fig. 5G and Supplementary Fig. S5D), whereas no binding was observed in HLA-A2<sup>-</sup> or TA<sup>-</sup> HNC cells (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,

**Figure 6.**

Cetuximab neoadjuvant therapy enhances HLA class I expression in patients' tumors in clinical responders but not in nonresponder HNC patients. A-D, tumor specimens from HNC patients ( $n = 24$ ) enrolled on UPCI trial 08-013 were biopsied pretreatment and after neoadjuvant treatment (cetuximab, i.v., 400 mg/m<sup>2</sup> day 1, then 250 mg/m<sup>2</sup> alone days 8, 15, and 22), and a tissue microarray was prepared from paired tumor biopsies. Expression of HLA-A (mAb HCA2), HLA-B (mAb HC10), and STAT1 (mAb C-24) was determined in neoadjuvant cetuximab-treated HNC patients by semiquantitative IHC, and correlated with tumor shrinkage (CT scan "responders") after 4 weeks of cetuximab therapy; \*,  $P \leq 0.05$ ; ns, not statistically significant.



both HLA alleles and STAT-1 were upregulated in the clinical responders ( $n = 7$ ) but not in nonresponders ( $n = 17$ ; Fig. 6A-D) to EGFR-specific mAb therapy.

## 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, IFN $\gamma$  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 IFN $\gamma$ -induced antigen presentation along with upregulated HLA alleles. Enhanced recognition of the peptide–HLA-A2 complex using the combination of cetuximab and IFN $\gamma$  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 EGF 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, IFN $\gamma$  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 STAT-1, HLA class I upregulation in cetuximab-treated patients in a novel neoadjuvant trial, suggesting that EGFR inhibition and/or IFN $\gamma$  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.

### Authors' Contributions

**Conception and design:** R.M. Srivastava, S. Ferrone, R.L. Ferris

**Development of methodology:** F. Concha-Benavente, R.R. Seethala, R.L. Ferris  
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** R.M. Srivastava, S. Trivedi, J. Hyun-bae, R.R. Seethala, B.F. Branstetter IV, R.L. Ferris

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** R.M. Srivastava, S. Trivedi, F. Concha-Benavente, J. Hyun-bae, L. Wang, R.R. Seethala, R.L. Ferris

**Writing, review, and/or revision of the manuscript:** R.M. Srivastava, B.F. Branstetter IV, S. Ferrone, R.L. Ferris

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** R.R. Seethala, B.F. Branstetter IV, R.L. Ferris

### Acknowledgments

The authors thank patients and their families for participating in this study; Clayton Mathis, Michael Meyer, and Bratislav Janjic, University of Pittsburgh Cancer Institute, for their excellent technical assistance; and the Ferris laboratory members for helpful suggestions.

### Grant Support

This work was supported by NIH grants R01 DE19727, P50 CA097190, and CA110249, and University of Pittsburgh Cancer Center support grant P30CA047904.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 24, 2015; revised April 6, 2015; accepted April 20, 2015; published OnlineFirst May 13, 2015.

### References

- Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006;354:567–78.
- Concha-Benavente F, Srivastava RM, Ferrone S, Ferris RL. EGFR-mediated tumor immunoevasion: the imbalance between phosphorylated STAT1 and phosphorylated STAT3. *Oncoimmunology* 2013;2:e27215.
- Pollack BP. EGFR inhibitors, MHC expression and immune responses: can EGFR inhibitors be used as immune response modifiers? *Oncoimmunology* 2012;1:71–74.
- Leone P, Shin EC, Perosa F, Vacca A, Dammacco F, Racanelli V. MHC class I antigen processing and presenting machinery: organization, function, and defects in tumor cells. *J Natl Cancer Inst* 2013;105:1172–87.
- Ferris RL, Hunt JL, Ferrone S. Human leukocyte antigen (HLA) class I defects in head and neck cancer: molecular mechanisms and clinical significance. *Immunol Res* 2005;33:113–33.
- Ferris RL, Whiteside TL, Ferrone S. Immune escape associated with functional defects in antigen-processing machinery in head and neck cancer. *Clin Cancer Res* 2006;12:3890–5.
- Herrmann F, Lehr HA, Drexler I, Sutter G, Hengstler J, Wollscheid U, et al. HER-2/neu-mediated regulation of components of the MHC class I antigen-processing pathway. *Cancer Res* 2004;64:215–20.
- Nouri A, Cannell H, Onguti M, Tezabwala B, Oliver R. The possible relevance of the expression of MHC antigens and of EGF receptor in aggressive oral tumours. *Int J Oncol* 1997;10:1217–22.
- Seliger B. Molecular mechanisms of MHC class I abnormalities and APM components in human tumors. *Cancer Immunol Immunother* 2008;57:1719–26.
- Mimura K, Shiraiishi K, Mueller A, Izawa S, Kua LF, So J, et al. The MAPK pathway is a predominant regulator of HLA-A expression in esophageal and gastric cancer. *J Immunol* 2013;191:6261–72.

11. Lopez-Albaitero A, Nayak JV, Ogino T, Machandia A, Gooding W, DeLeo AB, et al. Role of antigen-processing machinery in the in vitro resistance of squamous cell carcinoma of the head and neck cells to recognition by CTL. *J Immunol* 2006;176:3402–9.
12. Trivedi S, Concha-Benavente F, Srivastava RM, Jie HB, Gibson SP, Schmitt NC, et al. Immune biomarkers of anti-EGFR monoclonal antibody therapy. *Ann Oncol* 2014;26:40–7.
13. Leibowitz MS, Andrade Filho PA, Ferrone S, Ferris RL. Deficiency of activated STAT1 in head and neck cancer cells mediates TAP1-dependent escape from cytotoxic T lymphocytes. *Cancer Immunol Immunother* 2011;60:525–35.
14. Pollack BP, Sapkota B, Cartee TV. Epidermal growth factor receptor inhibition augments the expression of MHC class I and II genes. *Clin Cancer Res* 2011;17:4400–13.
15. Lupberger J, Duong FH, Fofana I, Zona L, Xiao F, Thumann C, et al. Epidermal growth factor receptor signaling impairs the antiviral activity of interferon-alpha. *Hepatology* 2013;58:1225–35.
16. Fletcher EV, Love-Homan L, Sobhakumari A, Feddersen CR, Koch AT, Goel A, et al. EGFR inhibition induces proinflammatory cytokines via NOX4 in HNSCC. *Mol Cancer Res* 2013;11:1574–84.
17. Leibowitz MS, Srivastava RM, Andrade Filho PA, Egloff AM, Wang L, Seethala RR, et al. SHP2 is overexpressed and inhibits pSTAT1-mediated APM component expression, T-cell attracting chemokine secretion, and CTL recognition in head and neck cancer cells. *Clin Cancer Res* 2013;19:798–808.
18. Srivastava RM, Lee SC, Andrade Filho PA, Lord CA, Jie HB, Davidson HC, et al. Cetuximab-activated natural killer and dendritic cells collaborate to trigger tumor antigen-specific T-cell immunity in head and neck cancer patients. *Clin Cancer Res* 2013;19:1858–72.
19. Lee SC, Srivastava RM, Lopez-Albaitero A, Ferrone S, Ferris RL. Natural killer (NK): dendritic cell (DC) cross talk induced by therapeutic monoclonal antibody triggers tumor antigen-specific T cell immunity. *Immunol Res* 2011;50:248–54.
20. Zhao M, Sano D, Pickering CR, Jasser SA, Henderson YC, Clayman GL, et al. Assembly and initial characterization of a panel of 85 genomically validated cell lines from diverse head and neck tumor sites. *Clin Cancer Res* 2011;17:7248–64.
21. Heo DS, Snyderman C, Gollin SM, Pan S, Walker E, Deka R, et al. Biology, cytogenetics, and sensitivity to immunological effector cells of new head and neck squamous cell carcinoma lines. *Cancer Res* 1989;49:5167–75.
22. Bandoh N, Ogino T, Cho HS, Hur SY, Shen J, Wang X, et al. Development and characterization of human constitutive proteasome and immunoproteasome subunit-specific monoclonal antibodies. *Tissue Antigens* 2005;66:185–94.
23. Frank DA, Mahajan S, Ritz J. Fludarabine-induced immunosuppression is associated with inhibition of STAT1 signaling. *Nat Med* 1999;5:444–7.
24. Fryknes M, Dhar S, Oberg F, Rickardson L, Rydaker M, Goransson H, et al. STAT1 signaling is associated with acquired crossresistance to doxorubicin and radiation in myeloma cell lines. *Int J Cancer* 2007;120:189–95.
25. Mitra RS, Nickoloff BJ. Epidermal growth factor and transforming growth factor-alpha decrease gamma interferon receptors and induction of intercellular adhesion molecule (ICAM-1) on cultured keratinocytes. *J Cell Physiol* 1992;150:264–8.
26. Kohrt HE, Colevas AD, Houot R, Weiskopf K, Goldstein MJ, Lund P, et al. Targeting CD137 enhances the efficacy of cetuximab. *J Clin Invest* 2014;124:2668–82.
27. Lopez-Albaitero A, Lee SC, Morgan S, Grandis JR, Gooding WE, Ferrone S, et al. Role of polymorphic Fc gamma receptor IIIa and EGFR expression level in cetuximab mediated, NK cell dependent in vitro cytotoxicity of head and neck squamous cell carcinoma cells. *Cancer Immunol Immunother* 2009;58:1853–64.
28. Yewdell JW, Reits E, Neeffes J. Making sense of mass destruction: quantitating MHC class I antigen presentation. *Nat Rev Immunol* 2003;3:952–61.
29. Johnson DR. Locus-specific constitutive and cytokine-induced HLA class I gene expression. *J Immunol* 2003;170:1894–902.
30. Balsamo M, Vermi W, Parodi M, Pietra G, Manzini C, Queirolo P, et al. Melanoma cells become resistant to NK-cell-mediated killing when exposed to NK-cell numbers compatible with NK-cell infiltration in the tumor. *Eur J Immunol* 2012;42:1833–42.
31. Jamil KM, Khakoo SI. KIR/HLA interactions and pathogen immunity. *J Biomed Biotechnol* 2011:298348.
32. Neisig A, Wubbolts R, Zang X, Melief C, Neeffes J. Allele-specific differences in the interaction of MHC class I molecules with transporters associated with antigen processing. *J Immunol* 1996;156:3196–206.
33. Neeffes JJ, Ploegh HL. Allele and locus-specific differences in cell surface expression and the association of HLA class I heavy chain with beta 2-microglobulin: differential effects of inhibition of glycosylation on class I subunit association. *Eur J Immunol* 1988;18:801–10.
34. Wang K, Heron DE, Flickinger JC, Rwigema JC, Ferris RL, Kubicek GJ, et al. A retrospective, deformable registration analysis of the impact of PET-CT planning on patterns of failure in stereotactic body radiation therapy for recurrent head and neck cancer. *Head & Neck Oncol* 2012;4:12.
35. Andrade Filho PA, Ito D, Deleo AB, Ferris RL. CD8+ T cell recognition of polymorphic wild-type sequence p53(65–73) peptides in squamous cell carcinoma of the head and neck. *Cancer Immunol Immunother* 2010;59:1561–8.
36. Andrade Filho PA, Lopez-Albaitero A, Gooding W, Ferris RL. Novel immunogenic HLA-A\*0201-restricted epidermal growth factor receptor-specific T-cell epitope in head and neck cancer patients. *J Immunother* 2010;33:83–91.
37. Lopez-Albaitero A, Mailliard R, Hackman T, Andrade Filho PA, Wang X, Gooding W, et al. Maturation pathways of dendritic cells determine TAP1 and TAP2 levels and cross-presenting function. *J Immunother* 2009;32:465–73.
38. Oliveras-Ferraro C, Vazquez-Martin A, Queralt B, Adrados M, Ortiz R, Cufi S, et al. Interferon/STAT1 and neuregulin signaling pathways are exploratory biomarkers of cetuximab (Erbix(R)) efficacy in KRAS wild-type squamous carcinomas: a pathway-based analysis of whole human-genome microarray data from cetuximab-adapted tumor cell-line models. *Int J Oncol* 2011;39:1455–79.
39. Garrido C, Rabasa A, Garrido C, Lopez A, Chao L, Garcia-Lora AM, et al. Preclinical modeling of EGFR-specific antibody resistance: oncogenic and immune-associated escape mechanisms. *Oncogene* 2013;33:3129–39.
40. Chatterjee-Kishore M, Kishore R, Hicklin DJ, Marincola FM, Ferrone S. Different requirements for signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 in the regulation of low molecular mass polypeptide 2 and transporter associated with antigen processing 1 gene expression. *J Biol Chem* 1998;273:16177–83.
41. Chatterjee-Kishore M, Wright KL, Ting JP, Stark GR. How Stat1 mediates constitutive gene expression: a complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene. *EMBO J* 2000;19:4111–22.
42. Akbay EA, Koyama S, Carretero J, Altabel A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discov* 2013;3:1355–63.