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## Comment on “Tumor-initiating cells establish an IL-33–TGF- $\beta$ niche signaling loop to promote cancer progression”

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1 **Title: Comment on “Tumor-initiating cells establish an IL-33–TGF- $\beta$  niche**  
2 **signaling loop to promote cancer progression”**

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32 **Abstract:**

33 Taniguchi *et al.* (Research Article, 17 July 2020, eaay1813) claim that the cytokine IL-33 induces  
34 accumulation of tumor-associated macrophages expressing the IgE receptor FcεRI. While these  
35 findings hold great therapeutic promise, we provide evidence that the anti-FcεRI antibody used in  
36 this study is not specific for FcεRI on macrophages, which raises concerns about the validity of  
37 some of the conclusions.

38

39

40 **Main Text:**

41

42 The recent article by Taniguchi *et al.* (1) showed that IL-33 induces the accumulation of a subset  
43 of tumor-associated macrophages promoting malignant progression of squamous cell carcinoma.

44 A major claim of the study is that this macrophage population is characterized by expression of  
45 the high-affinity IgE receptor (FcεRI). However, concerns have been raised about the specificity  
46 of the anti-mouse FcεRI antibody (clone MAR-1) used by the authors, especially the potential of  
47 this clone to cross-react with IgG Fcγ receptors (FcγRs) (2).

48

49 FcεRI is highly expressed by mast cells and basophils (3). Indeed, we found that MAR-1 stains  
50 peritoneal mast cells, as well as blood and spleen basophils in wild-type (WT) mice, but not in  
51 mice deficient for FcεRI (mFcεRI<sup>def</sup> mice) (**Fig. 1, A-C**). In stark contrast, MAR-1 stains  
52 peritoneal and spleen macrophages equally well in both WT and mFcεRI<sup>def</sup> mice (**Fig. 1, D and**  
53 **E**). These results indicate that in naïve mice, MAR-1 specifically recognizes FcεRI on mast cells  
54 and basophils, but labels macrophages in a FcεRI-independent manner.

55

56 In their article, Taniguchi *et al.* proposed that IL-33 induces expression of FcεRI in bone marrow-  
57 derived macrophages (1). We repeated these experiments using bone marrow cells from WT,  
58 mFcεRI<sup>def</sup>, and mFcγR<sup>null</sup> mice, which lack all mouse FcγRs (4). We confirmed that IL-33-induced  
59 macrophages derived from WT bone marrow were stained with MAR-1 (**Fig. 1F**). However, bone  
60 marrow-derived macrophages from mFcεRI<sup>def</sup> mice were stained to the same degree, further  
61 corroborating that MAR-1 staining of macrophages is not FcεRI-specific (**Fig. 1F**). Tang *et al.*

62 reported potential cross-reaction of MAR-1 with the FcγRs I and IV (2). In keeping with this, we  
63 found that IL-33-induced bone marrow-derived macrophages do express FcγRI and FcγRIV, and  
64 critically, that MAR-1 does not stain macrophages derived from mFcγR<sup>null</sup> bone marrow (**Fig. 1F**).  
65 Altogether, these results indicate that the MAR-1 staining observed in IL-33-induced bone-marrow  
66 derived macrophages relies solely on FcγR expression, and that these cells do not express FcεRI.

67

68 Finally, Taniguchi *et al.* purified ‘FcεRI<sup>+</sup>’ and ‘FcεRI<sup>neg</sup>’ macrophages from squamous cell  
69 carcinoma based on MAR-1 staining and analyzed their transcriptomes by RNA-seq (accession  
70 number GSE151783). In an attempt to clarify the identity of these populations in an unbiased,  
71 genome-wide manner, we compared their gene expression profiles to publicly available data from  
72 several highly purified immune cell populations (5), using the CIBERSORT method (6). This  
73 approach showed that both populations largely align to the macrophage/monocyte lineage  
74 signatures (**Fig. 1G**), confirming that the ‘FcεRI<sup>+</sup>’ cells are indeed macrophages. Even though cells  
75 were sorted based on MAR-1 staining, we detected virtually no *Fcer1a* mRNA signal for both  
76 MAR-1<sup>high</sup> and MAR-1<sup>low</sup> population in the authors’ dataset (**Fig. 1H**). By contrast, mRNA was  
77 abundant for all four FcγRs (FcγRI, IIB, III and IV) (**Fig. 1H**).

78

79 Based on these data, we conclude that the tumor-associated macrophage population described by  
80 Taniguchi *et al.* is unlikely to express FcεRI. To highlight the usefulness of MAR-1 labeling for  
81 the identification of these cells, but avoid confusion about functional implications, we suggest  
82 referring to them as ‘MAR-1<sup>high</sup> macrophages’ instead (a similar nomenclature is already used for  
83 monocyte-derived dendritic cells (7)). Although our in vitro data suggest that MAR-1 exclusively

84 binds FcγRs on macrophages (**Fig. 1F**), the lack of significant differences in FcγR mRNA between  
 85 MAR-1<sup>high</sup> and MAR-1<sup>low</sup> tumor-associated macrophages observed by RNAseq (**Fig. 1H**) suggests  
 86 that additional surface molecules might be involved in MAR-1 labeling in vivo. It will be important  
 87 to characterize further which surface molecules are recognized by MAR-1 in tumor-associated  
 88 macrophages, and whether these molecules represent potential targets for the development of  
 89 cancer treatments.

90

91 **References and Notes:**

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 109 place of the mouse *Fcεr1a* gene, thereby abrogating expression of mouse FcεRIα.  
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 112 10. ImmGen (<http://www.immgen.org>) Microarray Phase 2 data were downloaded from GEO  
 113 and normalized using Robust Multi-array Average (RMA) method. Six highly purified cell  
 114 populations (basophils, innate lymphoid cells [ILC], macrophages, mast cells, monocytes  
 115 and neutrophils) were selected and CIBERSORT was used to generate gene signatures for  
 116 each population. With these gene signatures, CIBERSORT was run to de-convolute the  
 117 MAR-1<sup>+</sup> and MAR-1<sup>neg</sup> cell populations purified by Taniguchi *et al* (GSE151783), and  
 118 generate fraction scores which were then visualized in stacked bar chart using R.  
 119

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134

135 **Competing interests:**

136 The authors declare that they have no competing interests.

137

138 **Author contributions:**

139 Experimental design, J.B.J.K, W.P.M.W, L.G, N.G, R.G, J.C. and L.L.R; Investigation, J.B.J.K,  
140 W.P.M.W, J.S., E.C., A.M, E.M, L.G., N.G, R.G., J.C. and L.L.R; Formal analysis, J.B.J.K,  
141 W.P.M.W, A.M, E.M, R.G, J.C. and L.L.R; Provided bone marrow cells from mFcyR<sup>null</sup> mice:

142 P.B; Writing (original draft), J.B.J.K, W.P.M.W and L.L.R; Writing (review and editing), all  
143 authors.

144 **Figure Legend:**

145

146 **Figure 1. Evidence that various macrophage populations, including IL-33-induced**  
147 **macrophages and tumor-associated macrophages, do not express FcεRI.**

148 (A–E) Quantification (mean fluorescence intensity [MFI]) of staining with MAR-1 or an isotype  
149 control antibody (Iso; Armenian hamster IgG) on peritoneal mast cells (F4/80<sup>-</sup>, CD115<sup>-</sup>, KIT<sup>+</sup>)  
150 (A), blood (B) and spleen (C) basophils (CD45<sup>int</sup>, CD131<sup>+</sup>), spleen macrophages (MΦ; Ly6G<sup>-</sup>,  
151 F4/80<sup>+</sup>) (D), and peritoneal macrophages (F4/80<sup>+</sup>, CD115<sup>+</sup>) (E) from wild-type (WT) and  
152 mFcεRI<sup>def</sup> mice (8). Results in A–E show values from individual mice; bars indicate means ± SEM  
153 pooled from three (B and C) or four (A, D, and E) independent experiments. \*\*,  $P < 0.01$ ; \*\*\*,  $P$   
154  $< 0.001$  by Mann–Whitney  $U$  test. N.S. not significant ( $P > 0.05$ ). (F) Analysis of staining with  
155 MAR-1, an anti-FcγRI antibody (clone REA286), or an anti-FcγRIV antibody (clone 9E9) in IL-  
156 33-induced macrophages derived from KIT<sup>+</sup> bone marrow cells from WT, mFcεRI<sup>def</sup>, and  
157 mFcγR<sup>null</sup> mice following the protocol described by Taniguchi *et al.* (1) and Wilkinson *et al.* (9).  
158 Results are representative of three independent experiments. FMO: fluorescence minus one  
159 control. (G and H) Raw RNAseq data for MAR-1<sup>high</sup> and MAR-1<sup>low</sup> tumor-associated  
160 macrophages from Taniguchi *et al.* (1) were downloaded from GEO (GSE151783) and normalized  
161 to count per million (CPM) using the edgeR R package. (G) Reference gene expression signatures  
162 specific for different innate immune cell lineages (basophils, innate lymphoid cells [ILCs],  
163 macrophages, mast cells, monocytes, and neutrophils) were generated using publicly available  
164 ImmGen datasets from highly purified cell populations. The relative abundance of these reference  
165 signatures was then quantified in the gene expression data from MAR-1<sup>high</sup> and MAR-1<sup>low</sup> tumor-  
166 associated macrophages using CIBERSORT, to estimate the likelihood of these cells belonging to  
167 the macrophage lineage (6, 10). (H) *Fcer1a*, *Fcgr1*, *Fcgr2b*, *Fcgr3*, and *Fcgr4* RNA-seq data  
168 (CPM values) for MAR-1<sup>high</sup> and MAR-1<sup>low</sup> tumor-associated macrophages.



