

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

Jasper B J Kamphuis, William P M Worrall, Julien Stackowicz, Aurélie Mougel, Emilie Mauré, Eva Conde, Pierre Bruhns, Laurent Guilleminault, Nicolas Gaudenzio, Jinmiao Chen, et al.

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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:

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

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#### 40 Main Text:

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| 42 | The recent article by Taniguchi et al. (1) showed that IL-33 induces the accumulation of a subset       |
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| 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 (FceRI). However, concerns have been raised about the specificity        |
| 46 | of the anti-mouse FccRI antibody (clone MAR-1) used by the authors, especially the potential of         |
| 47 | this clone to cross-react with IgG Fc $\gamma$ receptors (Fc $\gamma$ Rs) (2).                          |
| 48 |   |
| 49 | FccRI 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 FcERI (mFcERIdef mice) (Fig. 1, A-C). In stark contrast, MAR-1 stains                |
| 52 | peritoneal and spleen macrophages equally well in both WT and mFccRI <sup>def</sup> mice (Fig. 1, D and |
| 53 | E). These results indicate that in naïve mice, MAR-1 specifically recognizes FcERI on mast cells        |
| 54 | and basophils, but labels macrophages in a FccRI-independent manner.                                    |
| 55 |   |
| 56 | In their article, Taniguchi et al. proposed that IL-33 induces expression of FccRI in bone marrow-      |
|    |   |

derived macrophages (1). We repeated these experiments using bone marrow cells from WT, mFccRI<sup>def</sup>, and mFc $\gamma$ R<sup>null</sup> mice, which lack all mouse Fc $\gamma$ Rs (4). We confirmed that IL-33-induced macrophages derived from WT bone marrow were stained with MAR-1 (**Fig. 1F**). However, bone marrow-derived macrophages from mFccRI<sup>def</sup> mice were stained to the same degree, further corroborating that MAR-1 staining of macrophages is not FccRI-specific (**Fig. 1F**). Tang *et al.* 

| 62 | reported potential cross-reaction of MAR-1 with the FcyRs I and IV (2). In keeping with this, we           |
|----|--|
| 63 | found that IL-33-induced bone marrow-derived macrophages do express FcyRI and FcyRIV, and                  |
| 64 | critically, that MAR-1 does not stain macrophages derived from $mFc\gamma R^{null}$ 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 FcyR expression, and that these cells do not express FccRI.           |
| 67 |  |
| 68 | Finally, Taniguchi et al. purified 'FceRI+' and 'FceRIneg' 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                     |

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

abundant for all four FcyRs (FcyRI, IIB, III and IV) (Fig. 1H).

signatures (Fig. 1G), confirming that the 'FceRI<sup>+</sup>' cells are indeed macrophages. Even though cells

were sorted based on MAR-1 staining, we detected virtually no Fcerla mRNA signal for both

MAR-1<sup>high</sup> and MAR-1<sup>low</sup> population in the authors' dataset (Fig. 1H). By contrast, mRNA was



| 84   | binds   | Fc $\gamma$ Rs on macrophages ( <b>Fig. 1F</b> ), the lack of significant differences in Fc $\gamma$ R 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 ad | ditional surface molecules might be involved in MAR-1 labeling in vivo. It will be important  |
| 87   | to cha  | racterize further which surface molecules are recognized by MAR-1 in tumor-associated   |
| 88   | macro   | phages, and whether these molecules represent potential targets for the development of  |
| 89   | cancer  | treatments.   |
| 90   |         |   |
| 91   | Refer   | ences and Notes:  |
| 92   | 1.      | S. Taniguchi <i>et al.</i> , Tumor-initiating cells establish an IL-33-TGF-beta niche signaling loop  |
| 93<br>94<br>95                                       | 2.      | to promote cancer progression. <i>Science</i> <b>369</b> , (2020).<br>X. Z. Tang, J. B. Jung, C. D. C. Allen, A case of mistaken identity: The MAR-1 antibody<br>to mouse FcepsilonRIalpha cross-reacts with FcgammaRI and FcgammaRIV. <i>J Allergy</i>   |
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| 101<br>102<br>103                                    | 5.      | T. S. Heng, M. W. Painter, C. Immunological Genome Project, The Immunological Genome Project: networks of gene expression in immune cells. <i>Nat Immunol</i> <b>9</b> , 1091-1094 (2008).  |
| 104<br>105   | 6.      | A. M. Newman <i>et al.</i> , Robust enumeration of cell subsets from tissue expression profiles.<br><i>Nat Methods</i> <b>12</b> , 453-457 (2015).  |
| 106<br>107<br>108                                    | 7.      | M. Plantinga <i>et al.</i> , Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. <i>Immunity</i> <b>38</b> , 322-335 (2013).  |
| 109<br>110   | 8.      | We used hFccRI $\alpha^{KI}$ mice from Genoway, in which the human <i>Fcer1a</i> gene is inserted in place of the mouse <i>Fcer1a</i> gene, thereby abrogating expression of mouse FccRI $\alpha$ .   |
| 111<br>112   | 9.      | A. C. Wilkinson <i>et al.</i> , Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation <i>Nature</i> <b>571</b> 117-121 (2019)  |
| 112<br>113<br>114<br>115<br>116<br>117<br>118<br>119 | 10.     | ImmGen ( <u>http://www.immgen.org</u> ) Microarray Phase 2 data were downloaded from GEO and normalized using Robust Multi-array Average (RMA) method. Six highly purified cell populations (basophils, innate lymphoid cells [ILC], macrophages, mast cells, monocytes and neutrophils) were selected and CIBERSORT was used to generate gene signatures for each population. With these gene signatures, CIBERSORT was run to de-convolute the MAR-1 <sup>+</sup> and MAR-1 <sup>neg</sup> cell populations purified by Taniguchi <i>et al</i> (GSE151783), and generate fraction scores which were then visualized in stacked bar chart using R. |



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| 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 mFcγR<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
- authors.

144 **Figure Legend:** 

145

## 146Figure 1. Evidence that various macrophage populations, including IL-33-induced147macrophages and tumor-associated macrophages, do not express FceRI.

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>) (A), blood (B) and spleen (C) basophils (CD45<sup>int</sup>, CD131<sup>+</sup>), spleen macrophages (M $\Phi$ ; Ly6G<sup>-</sup>, 150 151 F4/80<sup>+</sup>) (D), and peritoneal macrophages (F4/80<sup>+</sup>, CD115<sup>+</sup>) (E) from wild-type (WT) and mFc $\in$ RI<sup>def</sup> mice (8). Results in A–E show values from individual mice; bars indicate means  $\pm$  SEM 152 pooled from three (B and C) or four (A, D, and E) independent experiments. \*\*, P < 0.01; \*\*\*, P 153 154 < 0.001 by Mann–Whitney U test. N.S. not significant (P > 0.05). (F) Analysis of staining with 155 MAR-1, an anti-FcyRI antibody (clone REA286), or an anti-FcyRIV antibody (clone 9E9) in IL-33-induced macrophages derived from KIT+ bone marrow cells from WT, mFccRIdef, and 156 mFc $\gamma$ R<sup>null</sup> mice following the protocol described by Taniguchi *et al.* (1) and Wilkinson *et al.* (9). 157 Results are representative of three independent experiments. FMO: fluorescence minus one 158 control. (G and H) Raw RNAseq data for MAR-1<sup>high</sup> and MAR-1<sup>low</sup> tumor-associated 159 160 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 ImmGen datasets from highly purified cell populations. The relative abundance of these reference 164 signatures was then quantified in the gene expression data from MAR-1<sup>high</sup> and MAR-1<sup>low</sup> tumor-165 166 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 (CPM values) for MAR-1<sup>high</sup> and MAR-1<sup>low</sup> tumor-associated macrophages. 168

