Combined effect of anti-BAG3 and anti-PD-1 treatment on macrophage infiltrate, CD8$^+$ T cell number and tumour growth in pancreatic cancer

We read with great interest the article by Zhang *et al*[1] showing that CD8$^+$ cell infiltration in pancreatic tumours can be enhanced by depletion of myeloid cells (CD11b$^+$ macrophages and myeloid-derived

suppressor cells) and that the depletion of CD11b$^+$ cells resulted in decreased PD-L1 expression on cancer cells thus impairing the triggering of the inhibitory receptor PD-1 on T cells.[1] Recruitment and activation of CD8$^+$ lymphocytes in tumours are suppressed by mechanisms only partially understood and rescuing CD8$^+$ cell infiltrate in tumours is one of the objectives of immunotherapies.[1 2] Tumour-associated macrophages (TAMs) play a crucial role in the relation between tumour cells and their environment.[3] Here, we confirm the interplay between macrophages and CD8$^+$ cells in pancreatic cancer and identify a potential way to exploit this enhancing effect of anti-PD-1 treatment. Indeed, we show that reduction of macrophage infiltrate, through treatment with an anti-Bcl-2-Associated athanoGene 3 (BAG3) antibody,[4] results in increased number of CD8$^+$ cells in pancreatic tumours in a murine model. BAG3 is a co-chaperone of the heat shock protein 70 whose expression is induced in response to stress but has been shown to be constitutive in cancers including pancreatic ductal adenocarcinomas (PDAC). We recently showed that BAG3 is secreted by PDAC cells and binds to TAMs, inducing their activation and the release of factors that support tumour growth and metastatic spreading. Treatment of PDAC-carrying mice with an anti-BAG3 mAb that interacts with a portion of BAG3 protein (from aa 385 to aa 399) not overlapping the BAG domain resulted in reduced tumour growth and metastatic spreading; analysis of tumour biopsies from anti-BAG3-treated mice showed a marked reduction of TAMs and a decrease in macrophage-released cytokines.[4 5]

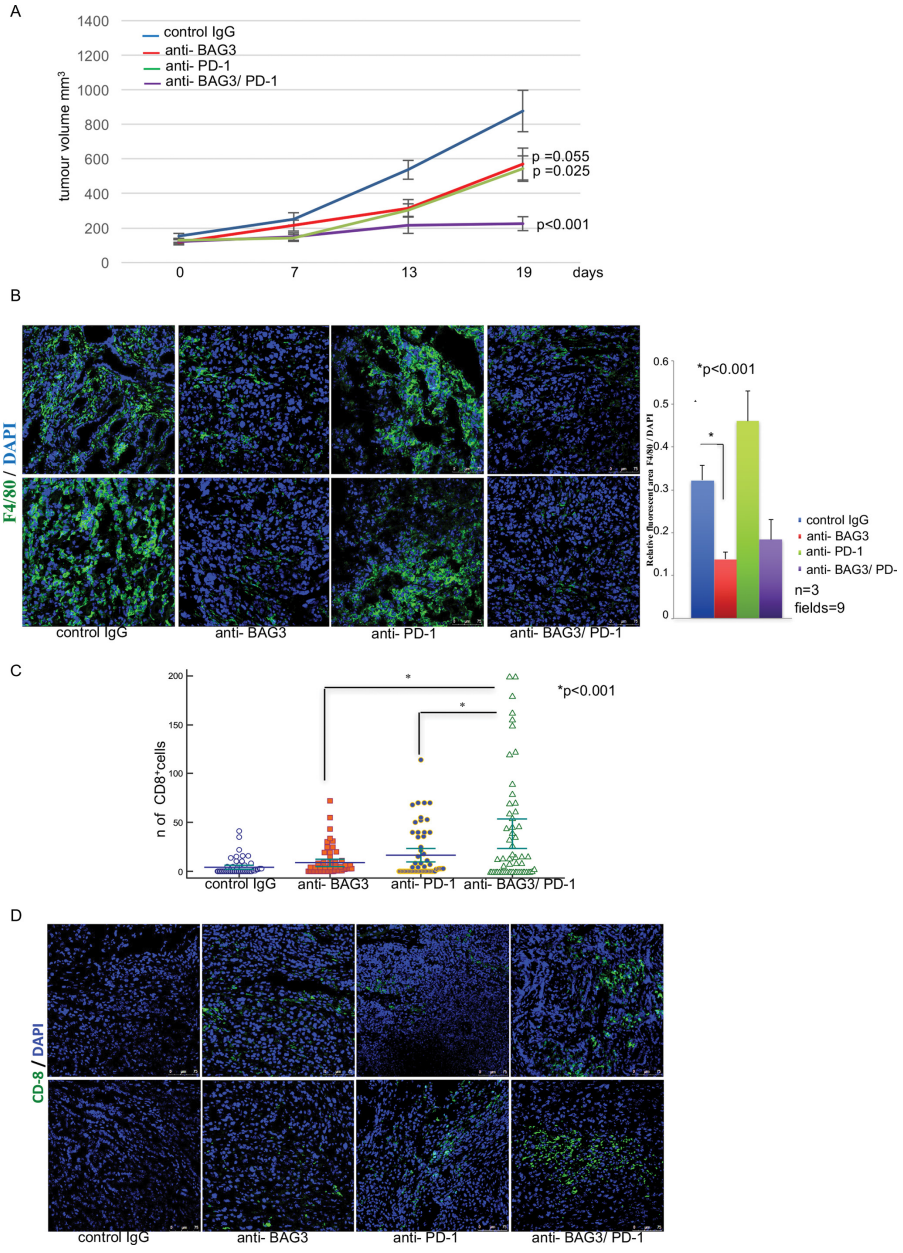

Figure 1 Combined effect of anti-Bcl-2-Associated athanoGene 3 (anti-BAG3) and anti-PD-1 antibodies in inhibiting pancreatic ductal adenocarcinomas growth and increasing the number of CD8$^+$ cells in tumours. (A) Tumour growth of syngeneic grafted tumours treated as indicated. (B) Analysis of macrophage infiltrate in tumours by F480 staining. (C) Count of CD8$^+$ infiltrating lymphocytes in tumours. (D) Representative images of CD8 positivity in excised tumours of the four different groups. For more details see online supplementary information.

Now we used the anti-BAG3 mAb in combination with an anti-PD-1 antibody to treat mice PDAC allografts. To this end, murine Kras-driven pancreatic cancer cells (mt4-2D)[6] were subcutaneously grafted in syngeneic immunocompetent (C57BL6) mice[4] and when tumours size reached about 100 mm^3 mice were treated for 19 days with an anti-BAG3 mAb, an anti-PD-1 antibody or a combination of the two. Histochemical staining showed that tumours appeared as low differentiated pancreatic adenocarcinoma; as expected for tumours derived from K-Ras-mutant cells, all tumour showed high expression of phospho-ERK (online supplementary figure 1) Treatment with either anti-BAG3 mAb or anti-PD-1 antibody resulted in a significant reduction of tumour growth that was even more impressive when the two antibodies were used in combination, suggesting that the block of BAG3 activity was additive with the block of PD-1 (figure 1A) as demonstrated by the not significant interaction term in a two-way analysis of variance (online supplementary figure 2). As previously reported, treatment with anti-BAG3 mAb resulted in reduced macrophage infiltrate[4] that on the contrary appeared to be increased in anti-PD-1-treated animals (figure 1B). Interestingly, the macrophage infiltrate was also reduced in the animals treated with the combination of the two antibodies. Analysis of tumour sections showed that CD8$^+$ cells were hardly detectable in control tumours, while their number was increased following treatment with anti-BAG3 or anti-PD-1 antibody and a higher effect was observed in mice treated with the two in combination (figure 1C, D).

In conclusion, blocking BAG3 activity results in an increased number of CD8$^+$ cells, with potential antitumour effects. Such increase is likely to be due, at least in part, to the decrease of TAMs-derived factors[4] that suppress CD8$^+$ lymphocytes influx or subsistence in tumour tissues. Whether BAG3 also impacts on other regulatory circuits requires further investigation, nevertheless, our observations disclose a BAG3-mediated mechanism that suppresses CD8$^+$ cell recruitment. Furthermore, our findings

indicate the potential effectiveness of anti-BAG3-directed and anti-PD-1-directed strategies in fighting pancreatic cancer.

Vittoria Iorio,[1,2] Alessandra Rosati,[1,2] Raffaella D'Auria,[1,2] Margot De Marco,[1,2] Liberato Marzullo,[1,2] Anna Basile,[1,2] Michelina Festa,[1,3] Maria Pascale,[1,3] Paolo Remondelli,[2] Mario Capunzo,[2] Gianluca Sala,[4] Verena Damiani,[4] Giuseppina Amodio,[2] Marta Di Nicola,[4] Rossano Lattanzio,[4] Maria Caterina Turco,[1,2] Vincenzo De Laurenzi[1,4]

[1]Biouniversa srl, c/o University of Salerno, Montoro, Avellino, Italy
[2]Department of Medicine, Surgery and Dentistry, Schola Medica Salernitana, University of Salerno, Baronissi, Salerno, Italy
[3]Department of Pharmacy, Division of Biomedicine 'A Leone', University of Salerno, Fisciano, Salerno, Italy
[4]Department of Medicine and Biotechnology, University G d'Annunzio and CeSI-MeT, Chieti, Italy

Correspondence to Professor Maria Caterina Turco, Department of Medicine and Surgery, Schola Medica Salernitana, University of Salerno, Baronissi, Salerno 84081, Italy; mcturco@unisa.it

Acknowledgements This work was supported in part by Associazione Italiana per la Ricerca sul Cancro (AIRC IG-14701) to MCT (AIRC IG-15196), VDL and (AIRC IG-18467) GS and by Ministero dell' Istruzione, dell'Università e della Ricerca (PRIN 2012CK5RPF_003). We acknowledge Professor Dave Tuveson for kindly providing murine Kras-driven pancreatic cancer cell line (mt4-2D).

Contributors VI, AR, MCT and VDL were involved in the study concept and design and drafting and revision of the manuscript. VI, AR, RDA, MDM, LM, AB, MF, MP, MC, GS, MDN, RL and VD were involved in acquisition of data, analysis, interpretation of data and statistical analysis. PR and GA provided technical support.

Competing interests AR, MDM, LM, AB, MF, MP, VDL and MCT are shareholders of the Academic Spin-off BIOUNIVERSA that provided anti-BAG3 antibodies. The remaining authors declare no competing financial interests.

Provenance and peer review Not commissioned; externally peer reviewed.

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2017-314225).

VI and AR contributed equally.

To cite Iorio V, Rosati A, D'Auria R, *et al*. *Gut* 2018;**67**:780–782.

Received 28 March 2017
Revised 26 July 2017
Accepted 29 July 2017
Published Online First 11 August 2017

Gut 2018;**67**:780–782. doi:10.1136/gutjnl-2017-314225

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