

However, as radiation-associated lymphopenia is common and long-lasting in patients with glioblastoma, as well as in patients with pancreatic, lung, and breast cancer, where dexamethasone is not an integral part of therapy, it is likely that the immunosuppression described by Dr Wong *et al* was due to prior radiation exposure, rather than to dexamethasone treatment. At a minimum, this issue should be formally addressed in this manuscript and in subsequent work regarding this important topic.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Response to: Comment on 'Dexamethasone exerts profound immunologic interference on treatment efficacy for recurrent glioblastoma'

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Sir,

We would like to thank you for an opportunity to respond to the comments from Drs Ellsworth and Grossman in their letter to the editor concerning our recent paper, 'Dexamethasone Exerts Profound Immunologic Interference on Treatment Efficacy for Recurrent Glioblastoma', by Wong *et al* (2015).

Contrary to the assertion by the authors, our paper did not claim that the effects of dexamethasone were mediated via steroid-induced lymphopenia. It is widely accepted that dexamethasone exerts pleiotropic effects on the immune system that lead to the suppression of multiple effector systems required for therapy-induced tumor rejection (Fauci, 1976; Benedetti *et al*, 2003). Within our single institution patient cohort, we aggressively weaned dexamethasone doses and we found that patient outcome correlated with T-cell counts. T-cell count was used as a marker of potential immunological competency to test if it correlated with outcome, as suggested by our initial observation in the phase III trial that high dexamethasone dose was correlated with a poorer survival. As pointed out by Drs Ellsworth and Grossman, the observed lymphocyte counts in our single institution cohort were probably related to patient treatment history, intrinsic immune state or both, but not necessarily to corticosteroid usage. Furthermore, overall survival as a function of the effect of dexamethasone in each of the two arms in the phase III trial was very likely independent of the T-lymphocyte counts of patients entering the trial, as supported by our single institution patient cohort where no correlation was observed between dexamethasone dose and T-lymphocyte count.

The authors also cited their work on the immunosuppressive effect of radiation and temozolomide when given to patients with newly diagnosed glioblastomas (Grossman *et al*, 2011). They found that 40% of patients had <200 CD4 cells mm⁻³ 2 months after initiation of treatment and this was associated with a poorer survival when compared with those with ≥200 CD4 cells mm⁻³. Given that corticosteroid use was not a controlled variable, it is possible that dexamethasone may have contributed to the poor survival outcome in this study. Regardless, the overall conclusion of their study was also consistent with our utilisation of T-lymphocyte counts as a marker of poor outcome. Furthermore, an earlier study by Hughes *et al* (2005) investigated the phenomenon of lymphopenia in the pre-temozolomide chemo-irradiation era and found that 24% of the cohort had <200 CD4 cells mm⁻³ whereas 76% had ≥200 CD4 cells mm⁻³. Therefore, it is possible that the addition of temozolomide to dexamethasone plus radiotherapy increased the proportion of patients who developed poor outcome and low CD4 lymphocyte count (from 24 to 40%). Taken together, it may be

important to re-examine the potential role of dexamethasone in these two studies.

Lastly, the authors also cited that treatment-related lymphopenia is a marker of poor outcome in pancreatic and non-small cell lung cancers (Balmanoukian *et al*, 2012; Campian *et al*, 2013; Tang *et al*, 2014; Wild *et al*, 2015). Our data are consistent with this contention, but do not address the cause of the low T-lymphocyte counts in our patients. It is notable that patients in these studies also received concurrent metagenetic chemotherapies, such as taxol/carboplatin, gemcitabine or gemcitabine/carboplatin, and dexamethasone was likely an important antiemetic in the premedication regimen and may therefore confound the outcome analysis.

Although it is hard to absolutely devolve the contribution of dexamethasone from prior radiation and chemotherapy effects in patients with recurrent glioblastoma, the NovoTTF-100A monotherapy arm in the phase III trial nevertheless offered us a unique opportunity to evaluate the sole effect of dexamethasone dosage because the influence of prior radiation and chemotherapy was randomized and balanced. In contrast to commonly used chemotherapeutic regimens (Grossman *et al*, 2011), NovoTTF-100A does not exert such deleterious effects on the immune system. Given these conditions, we were able to determine that subjects who received a dexamethasone dose of ≥4.1 mg day⁻¹ had a significantly shorter survival than those who took <4.1 mg day⁻¹. Therefore, one of the obvious implications of our work is that future clinical trials in the glioblastoma population may need to control for the confounding dexamethasone effect in outcome. Furthermore, it may be worthwhile to re-examine treatment outcomes of prior clinical trials based on dexamethasone stratification.

CONFLICT OF INTEREST

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BRAF-mutated metastatic colorectal cancer between past and future

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Sir,

We read with interest the meta-analysis by Rowland *et al* addressing the role of BRAF V600E mutation as predictor of benefit from anti-EGFR monoclonal antibodies (mAbs) in metastatic colorectal cancer.

Authors conclude that there is insufficient evidence to definitively state that RAS WT/BRAF MT individuals attain a reduced benefit from anti-EGFR mAbs compared with RAS WT/BRAF WT ones. Their conclusion is based on the lack of a significant interaction between BRAF mutational status and the effect of the addition of an anti-EGFR mAb to standard therapies (Rowland *et al*, 2015).

In our opinion some considerations are needed to properly put these results in the clinical perspective, as pointed out in our previous work (Pietrantonio *et al*, 2015).

First, it should be noted that in terms of PFS, where the confounding effect of subsequent lines of treatment is absent, the *P*-value for interaction is equal to 0.07. Of note, an alpha-error up to 0.10 is often considered reasonable for interaction tests. In any case, it should be considered that these analyses are based on the retrospective, unplanned evaluation of subgroups of patients included in randomized trials and are therefore definitely underpowered to evidence a statistically significant difference. Although the global number of patients included in the analysis is high, the low incidence of BRAF V600E mutation weakens the power of this analysis. In the meta-analysis, OS comparison included 3096 patients (89% BRAF wild-type and 11% BRAF mutated). Even if 100% of events had been observed – that is a clear overestimation, especially with respect to OS data – the statistical power to detect a significant interaction between BRAF mutational status and the effect of anti-EGFR mAbs (assuming hazard ratio 0.8 in BRAF wt and hazard ratio 1.0, that is, absence of effect, in BRAF mutant patients) would have been as low as about 50%. Therefore, even if the lack of statistical significance of the interaction test for OS is a matter of fact, the relevant risk of a false negative result should be properly acknowledged.

Second, results from FIRE-3 trial, comparing first-line FOLFIRI plus cetuximab with FOLFIRI plus bevacizumab were not included in the metanalysis by Rowland *et al*. In their discussion, authors elegantly argue that FIRE-3 is not sufficiently comparable to the other included trials, as bevacizumab use in the control arm is associated with a significant benefit, as compared with chemotherapy alone. We totally agree with that observation, but, again, by a practical perspective it should be recognised that first-line chemotherapy plus bevacizumab is one of the most common choices worldwide. From a clinical point of view, the decision of adding an anti-EGFR mAb to chemotherapy in patients with BRAF mutation, based on the absence of interaction between BRAF status and treatment efficacy, would be totally reasonable in the absence of therapeutic alternatives. Given that an alternative is actually available, the use of an anti-EGFR mAb, instead of bevacizumab, should be probably reserved to those patients who may actually derive benefit from these drugs, with a different and often less acceptable toxicity profile. To this purpose, the metanalysis by the same authors highlighting the role of panRAS mutations as predictors of resistance to anti-EGFR mAbs, also including results from FIRE-3, is of special interest (Pietrantonio *et al*, 2015; Sorich *et al*, 2015). Unfortunately, results in the RAS WT/BRAF WT subgroup of the FIRE-3 trial have not been provided yet, thus preventing from including this trial in the present analysis. As information about BRAF mutational status is also lacking from the other head-to-head randomized trials PEAK and CALGB80409, we recognise that the question about the 'best' biologic agent to be combined with a first-line chemotherapy doublet in BRAF mutant individuals is far from being answered.

Third, as BRAF mutant patients are often unable to receive subsequent lines of therapy (Seligmann *et al*, 2015), the choice of the upfront treatment is of paramount importance. Although results with doublets plus a biologic are disappointing (Stintzing *et al*, 2014), increasing evidences support the choice of FOLFOXIRI plus bevacizumab as a preferred option for fit patients (Fakih, 2015; Loupakis *et al*, 2014).

Nevertheless, more targeted approaches will hopefully enter the clinical scenario in the next future, based on promising results of early phase trials investigating BRAF ± MEK and EGFR inhibitors in molecularly selected patients (Atreya *et al*, 2015). Knowing BRAF status is today crucial to allow BRAF MT patients to enter clinical trials with those targeted agents.

In conclusion, although the negative predictive power of BRAF V600E mutation with respect to anti-EGFR mAbs will never be formally demonstrated in properly designed, wide and expensive clinical trials, BRAF testing is today recommended by major guidelines. In our opinion, irrespectively of the personal choice of treating physicians to expose BRAF mutant patients to anti-EGFR mAbs, BRAF clearly stands as a molecular marker able to inform clinical decisions in the daily practice, and hopefully its role in treatment decisions will be better defined in the near future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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