These results suggest that sarcomas with *MDM2* and/or *CDK4* amplification (both liposarcomas and intimal sarcomas) should be included for routine *NTRK* fusion testing. Above all, these results confirm that further studies remain essential to determine the frequency of *NTRK* gene fusions in different sarcoma subtypes and correlation with morphological, biological, and clinical features to better inform the optimal approach to NTRK gene fusion screening. Nevertheless, the functional significance was not clinically demonstrated in any of these three patients, since no patient received a TRK-fusion-inhibiting drug.

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DISCLOSURE

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Is impaired response to PD-1 blockers of high serum PD-1 patients related to immune complexes?

Ugurel et al.[1] reported in this journal that high serum programmed cell death protein 1 (sPD-1) levels were associated with lower clinical benefit in melanoma patients treated with PD-1 blockers, suggesting that sPD-1 binding to anti-PD-1 monoclonal antibody (mAb) might reduce the bioavailability of this therapeutic agent. However, the lack of obvious dose dependency in the clinical efficacy of PD-1 blockers does not entirely support the hypothesis that sPD-1-mediated loss of some antigenic binding sites might actually interfere with the antitumor activity of anti-PD-1 mAbs. Here we would like to raise the possibility that the process could also stem from the *in vivo* formation of sPD-1/anti-PD-1 mAb immune complexes (PD-1-IC). Indeed, as the immune complexes are stable antigen-antibody structures displaying distinctive regulatory functions through the interaction of mAb-fragment crystallizable (Fc) region with Fcγ-receptor-(FcγR)-expressing cells,[2] it is conceivable that PD-1-IC could decrease clinical benefit by inducing unwanted immunological effects via Fc/FcγR bonds.

We report here that PD-1 nivolumab IC (nivo-IC) can bind *in vitro* to all human FcγRs, including FcγRI/CD64, FcγRIIa/CD32a, FcγRIIb/CD32b, FcγRIIIa/CD16a and FcγRIIIb/CD16b, albeit with different kinetics. Rather, when nivo-IC is depleted of the Fc portion [$F(ab)_2$ nivo-IC], there is no interaction with any of the tested FcγRs (Figure 1A). To assess if the nivo-IC binding also occurred at the immune cell level leading to any functional consequences, we focused on human macrophages (hMϕ), which are $FcγRs^+$ elements (Figure 1B), highly represented in the tumor microenvironment.[3,4] As depicted in Figure 1C, hMϕ interact with nivo-IC in an fragment crystallizable receptor-dependent manner and rapidly internalize the complex as shown by confocal microscopy (Figure 1D). Most importantly, according to transcriptional (Figure 1E) and released cytokine analyses (Figure 1F), nivo-IC binding and endocytosis impair hMϕ polarization towards antitumor classically activated macrophages (M1) cells and switch them to a protumorigenic alternatively activated macrophages (M2) profile.

Although based only on *in vitro* data, this first report on the immunomodulating properties of PD-1-IC may generate new hypotheses about the mechanisms of resistance to PD-1 blockers (Figure 1G). Indeed, the presence of sPD-1 in cancer patients' blood,[1] the remarkable affinity of anti-PD-1 mAbs for their antigenic determinants and the abundance of FcγR-expressing myeloid cells in the tumor microenvironment[5] make the process described here likely to occur *in vivo* as well. If so, PD-1-IC could contribute to favor macrophage skewing toward protumorigenic cells and possibly hinder the cancer-associated myeloid cell

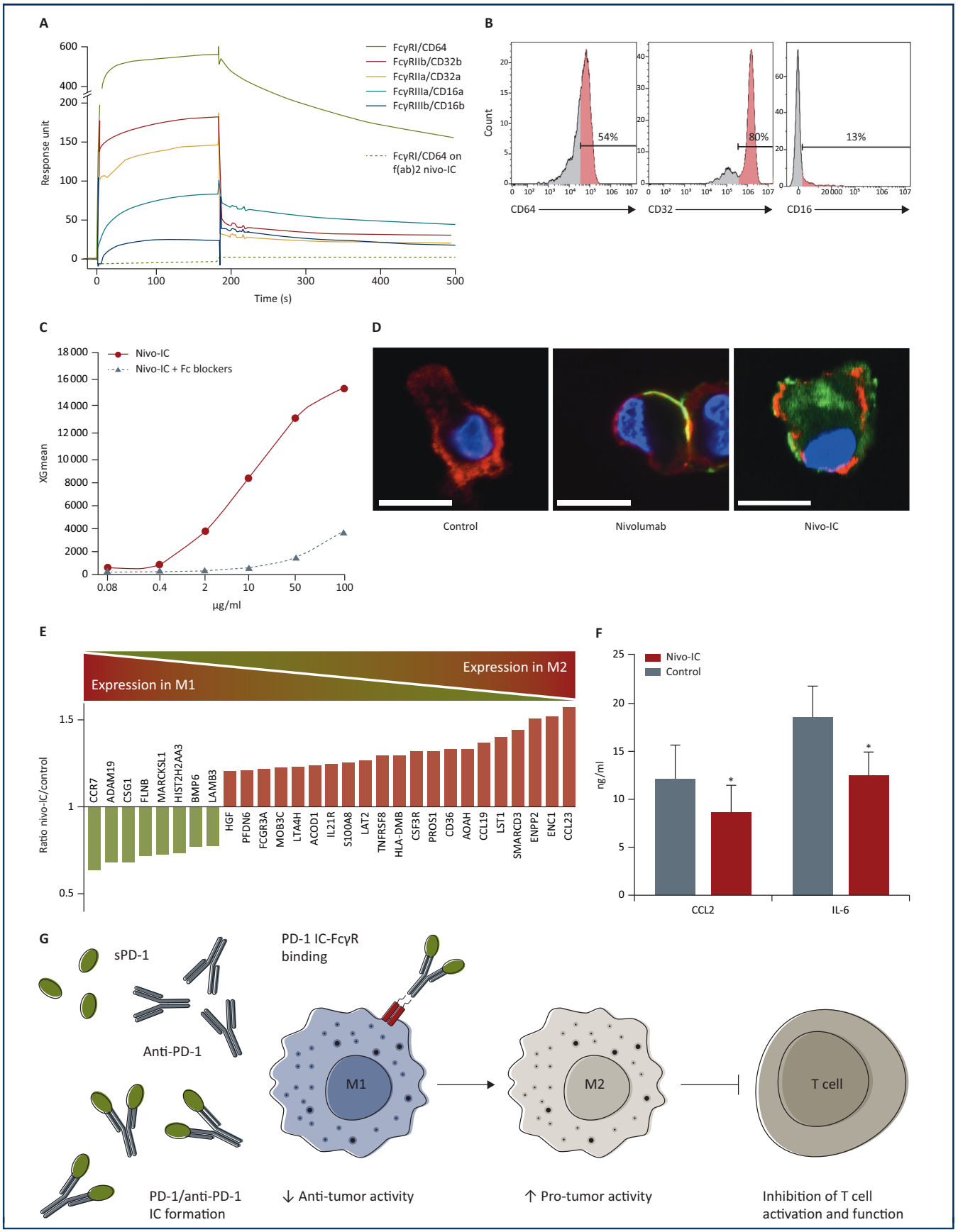

Figure 1. Nivo-IC-FcR binding and functional outcome in myeloid cells.
(A) Sensorgrams of FcγRI/CD64, FcγRIIa/CD32a, FcγRIIb/CD32b, FcγRIIIa/CD16a and FcγRIIIb/CD16b binding to nivo-IC (produced by coincubation of 2 : 1 molar ratio of antigen/antibody, without any single residual component). The lack of binding to $F(ab)_2$ nivo-IC of the FcγRI/CD64 (shown as representative of all the FcγRs tested) is reported as negative control. These experiments were generated by surface plasmon resonance (Biacore™ T200, GE Healthcare). (B-F) Human macrophages (hMΦ)

reprogramming recently described to precede tumor regression by PD-1 blockade.[3]

The findings depicted here build on our previous data indicating that M2-Mϕ tumor infiltrate correlates with poor clinical outcome in non-small-cell lung cancer (NSCLC) patients treated with PD-1 blockers and that F(ab)2-anti-PD1 mAb loses its pro-tumor effects in preclinical models.[4] Above all, our results suggest that tools of PD-1 blockade possibly avoiding Fc/FcγR interactions, such as mAbs null for fragment crystallizable receptor-binding[5] or tools for Ab-independent blockade, might contribute to potentiate clinical benefit of anti-PD-1 agents. Detecting PD-1-IC is technically challenging, as most anti-PD-1 mAbs recognize the active epitope seen by PD-1 blockers. Nevertheless, a test quantifying plasma PD-1-IC also promises to provide a new resistance biomarker to implement the predictive value of sPD-1.

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DISCLOSURE

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were differentiated from blood CD14^{+} monocytes by culture with macrophage colony-stimulating factor and tested for (B) the expression of CD64, CD32 and CD16 by flow cytometry (CytoFLEX S and Kaluza Software Analysis, Beckman Coulter). (C) *In vitro* binding of Alexa-488-conjugated PD-1-nivolumab immune complex (nivo-IC) with or without fragment crystallizable receptor (FcR) blocker pre-incubation (Miltenyi Biotec) analyzed by flow cytometry. (D) Cellular localization of Alexa-488-conjugated nivolumab and nivo-IC (green fluorescence) after incubation of hMΦ for 60 min, wheat germ agglutinin (red fluorescence) showing plasma membrane and 4′,6-diamidino-2-phenylindole (DAPI) (blue fluorescence) for nuclear DNA (scale bar 10 μm), were detected by confocal microscopy. (E) Transcriptional profiling (NanoString® Technologies, Seattle, WA) of hMΦ polarized to M1 macrophages with lipopolysaccharides and interferon gamma and concomitant treatment with or without nivolumab or nivo-IC. Normalized counts were analyzed using a two-tailed paired *t*-test. Genes with nominal *P* value <0.1 and IC/control ratios >1.2 or <0.8 were considered significant. (F) Cytokines released in 24-h supernatants were detected by Cytokine Bead Array (CBA) (BD™ Biosciences) (**P* value <0.05, paired *t*-test). All the above-mentioned data are representative of experiments repeated at least three times. (G) Hypothesized mechanism of activity of the PD-1-anti-PD-1 mAb IC on myeloid cells. High serum programmed cell death protein 1 (sPD-1) binds to anti-PD-1 mAb and the originating PD-1-IC interacts with the Fcγ receptors (FcγRs) expressed by macrophages, skewing them towards pro-tumorigenic cells and possibly interfering with antitumor T-cell immunity.