Supplementary Materials and Methods

Mice

Inbred C57BL/6 wild type (WT) mice, C57BL/6 p53+/-, and C57BL/6 CD11c DOG (DTR-QVA-eGFP) transgenic mice were bred and maintained at the Peter MacCallum Cancer Centre (Peter Mac) as described previously (1) (2). Mice 6-14 weeks old were used in all experiments. All experiments were performed in accordance with guidelines set out by the Peter Mac Animal Experimental Ethics Committee.

Tumor Models

Male mice were injected subcutaneously with low doses of MCA (Sigma Fine Chemicals) as indicated and described (3) and monitored for tumor development. At various time points after inoculation, MCA-treated mice bearing progressively growing primary fibrosarcomas were removed from the experiment (~10-20% of group)(1st stage). The remaining mice in each group were then treated weekly with either cIg or mAbs that deplete or block specific immune components for 3-6 weeks (2nd stage). After a 1-3 week break after the last immune intervention treatment, mice then received either cIg or anti-IFN-γ or a cocktail of anti-CD4/CD8/IFN-γ for a further 6 weeks (3rd stage). Mice were monitored for tumor development throughout for up to 600 days. Tumor size (cm²) for each individual mouse was recorded as described previously (3).

A cohort (n = 107) of male and female C57BL/6 p53+/- mice were aged and tumor burdened mice removed from the group prior to 750 days. Tumor free mice from 750 days were injected weekly for 8 weeks with mAbs as indicated. Mice were monitored
for the morbidity and the appearance of late-forming tumors for 200 days after the commencement of antibody treatment. A full autopsy was performed on all mice at sacrifice. Tumors were confirmed from H&E sections by a trained histopathologist, Dr. William Murray (Peter Mac).

**MAbs and cell depletions**

The majority of the anti-mouse mAbs used for neutralization or depletion have been previously described (3). These include control Igs (PIP, a mAb specific for bacterial glutathione S-transferase), anti-CD8α (YTS169.4), anti-CD4 (GK1.5) and anti-IFN-γ (H22). These were produced from hybridoma supernatants and purified in endotoxin-free form by Protein G affinity chromatography (Leinco Technologies, St. Louis, MO). Anti-AGP3 (control Ig (cIg), 4D2), anti-IL-23p19 (16E5), and anti-IL-12p40 (C17.8) were provided by AMGEN and have been described previously (4). CD11c+ cells were depleted in CD11c DOG transgenic mice using diptheria toxin A (DT) as previously described (2). 1A8 mAb produced in house was used to deplete Ly-6G+ neutrophils. Vehicle liposomes and clodrolip was used as previously described to deplete macrophages (2). We thank Dr Reto Swendener for the liposomal preparations.

**Statistical Analysis**

Significant differences in proportions of mice with tumors at each stage were determined by the Fishers Exact test. Differences in growth rate to cIg treated mice were determined by Mann-Whitney test. Values of p < 0.05 were considered significant.
References


