Supplementary Tables, Figures & Legends

Sitravatinib potentiates immune checkpoint blockade in refractory cancer models

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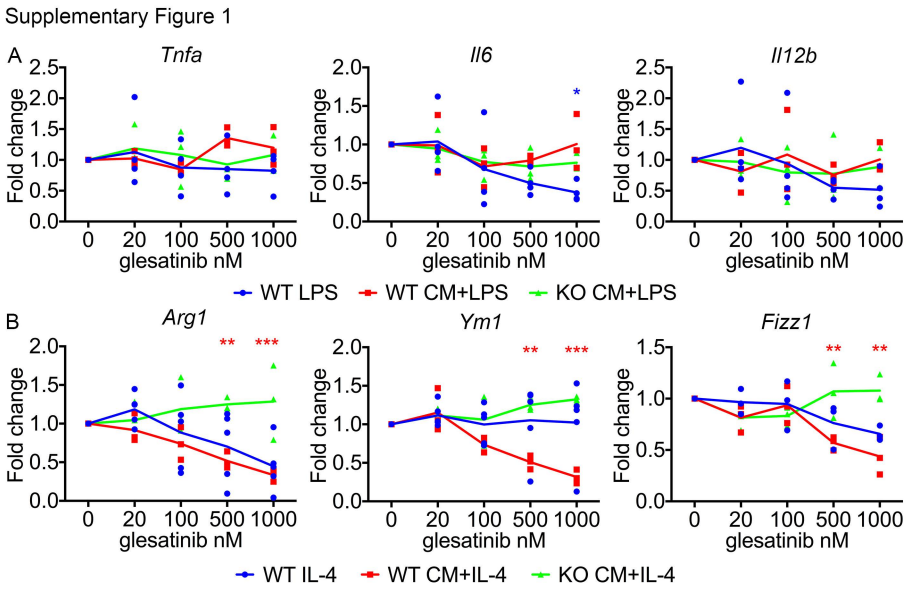
Supplementary Table 1. Pharmacokinetic analysis of sitravatinib in mice

Time (hr)	Total sitravatinib (ng/ml)	Total (nM)	Free (nM)
0	150	238	2.4
1	630	1000	10
4	710	1127	11.3
6	1300	2033	20.6
10	580	921	9.2
16	610	968	9.7
24	420	667	6.7

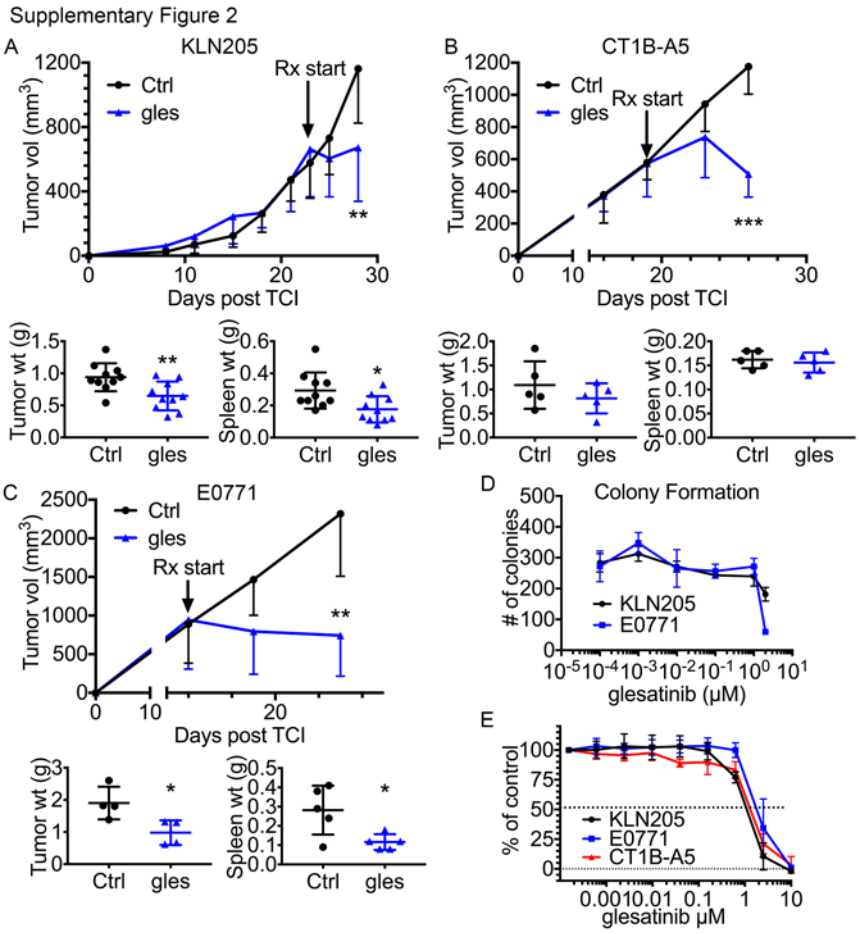
Mice were dosed at 20 mg/kg at time 0. Plasma was collected at the time points indicated and evaluated for total and free sitravatinib by mass spec analysis.

Supplementary Table 2. RT-qPCR primers

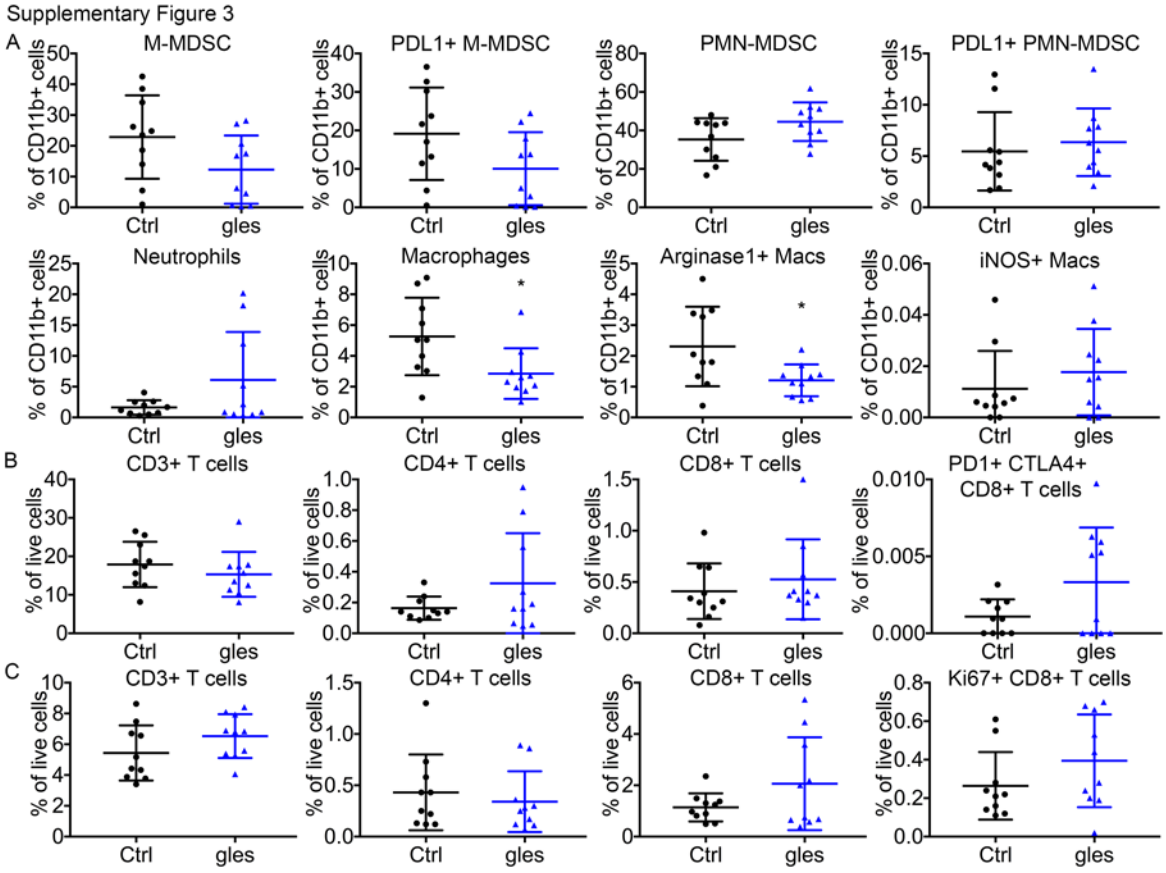
Gene	Forward (5'-3')	Reverse (5'-3')
Actb	CGGTTCCGATGCCCTGAGGCTCTT	CGTCACACTTCATGATGGAATTGA
Tnfa	TGTGAGGAAGGCTGTGCATT	GGTCAGGTTGCCTCTGTCTC
Il6	CGTGGAAATGAGAAAAGAGTTGTGC	TGGTACTCCAGAAGACCAGAGG
Il12b	AGCAGTAGCAGTTCCCCTGA	AGTCCCTTTGGTCCAGTGTG
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
YM1	TCTGGGTACAAGATCCCTGAA	TTTCTCCAGTGTAGCCATCCTT
Fizz1	CCCTTCTCATCTGCATCTCC	CTGGATTGGCAAGAAGTTCC



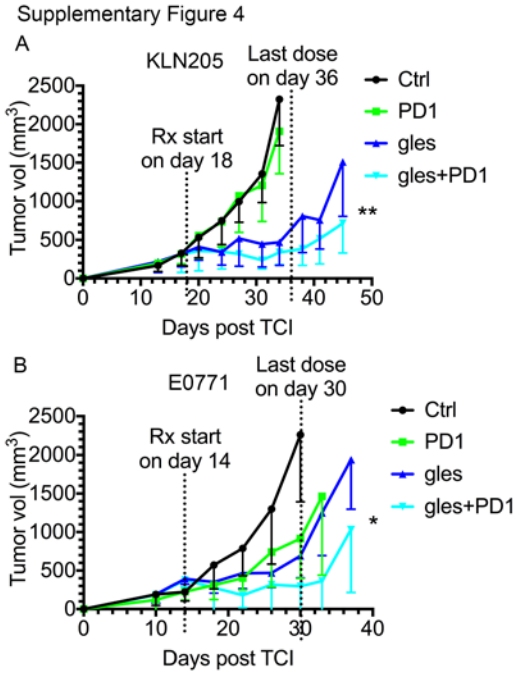
Supplementary Figure 1. MerTK inhibition with glesatinib directly affects macrophage phenotype. The expression of M1-type macrophage markers TNF-α, IL-6, and IL-12 (**A**) and M2-type macrophage markers arginase1, YM-1 and Fizz-1 (**B**) in bone marrow-derived macrophages (BMDMs). BMDMs were harvested from WT C57Bl/6 or *MerTK-/-* (green) mice, stimulated with 20 ng/ml LPS for 2 hours (**A**) or 40 ng/ml IL-4 for 18 hours (**B**). Each stimulation was performed +/- glesatinib (20, 100, 500, and 1000 nM) in the presence (red and green) or absence (blue) of conditioned media (CM) collected from KLN205 cells. The expression level of TNF-α, IL-6, IL-12, arginase1, YM-1, and Fizz-1 was determined by q-PCR. Three independent experiments using duplicate samples were performed. Data are displayed as fold change normalized to DMSO (0 nM) in each condition, mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.005 vs. DMSO in each condition by ANOVA.



Supplementary Figure 2. Glesatinib alone has potent antitumor activity in vivo. **A)**, **B)**, and **C)** In vivo assessment of treatment response of subcutaneously or orthotopically implanted tumors. We injected 0.5 x 10^6 KLN205 cells (**A**, n=11/group) subcutaneously into 6-week-old DBA/2 mice, 1 x 10^6 CT1B-A5 cells (**B**, n=5/group) were injected subcutaneously into 6-week-old C57BL/6 mice, and 0.5 x 10^6 E0771 cells (**C**, n=5/group) were injected orthotopically into the mammary fat pad of 6-week-old female C57BL/6 mice. Mice with established tumors (500-700 mm^3) were treated with control (Ctrl, vehicle, once per day) and glesatinib (gles, p.o. 60 mg/kg, q.d.). Effects on tumor growth are shown after 6 days of treatment. Tumor and spleen weight were determined in each mouse and was displayed as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.005 vs. control by t test. **D)** Colony formation for E0771 cell line grown in normal growth media ± glesatinib at the indicated doses for 14 days. Two independent experiments using triplicate samples were performed. Mean ± SD colonies/hpf are shown. **E)** Cell growth assays were performed in a 96-well format for 5 days using MTS. Three independent experiments using two 96-well plates/cell line were performed. Drug-sensitivity curves for 265 are displayed.



Supplementary Figure 3. Glesatinib alters the immune landscape of tumors to favor immune checkpoint blockade. Flow cytometry of tumor-associated myeloid (**A**) and lymphoid cells (**B**) from mice bearing KLN205 tumors treated for 6 days with glesatinib (gles, *n* = 9-10/group). Monocytic myeloid-derived suppressor cells (M-MDSCs; CD11b+ Ly6G- Ly6C+), PD-L1+ M-MDSCs, PMN-MDSCs (CD11b+ Ly6G+ Ly6C+), PD-L1+ PMN-MDSC, neutrophils (CD11b+ Ly6G+ Ly6C-), macrophages (CD11b+ Ly6G- Ly6C- F4/80+ CD11c+ MHCII+), Arg1+ macrophages (Macs), iNOS+ macrophages, CD3+ T cells, CD4+ T cells, CD8+ T cells, and PD1+ CTLA4+ CD8+ T cells were analyzed. **P* < 0.05 vs. control (Ctrl) by *t* test. **C)** Flow cytometry of splenocytes from mice bearing KLN205 tumors treated with glesatinib for 6 days (*n* = 9-10/group). CD3+ T cells, CD4+ T cells, CD8+ T cells and Ki67+ CD8+ T cells were analyzed. **P* < 0.05 vs. control by *t* test.



Supplementary Figure 4. Glesatinib enhances the efficacy of PD-1 blockade. A, B) In vivo assessment of treatment response of subcutaneously or orthotopically implanted tumors (*n* = 9-12/group) in combination with PD-1 blockade. We injected 0.5 x 10^6 KLN205 cells (**A**) subcutaneously into 6-week-old DBA/2 mice and 0.5 x 10^6 E0771 cells (**B**) were injected orthotopically into the mammary fat pad of 6-week-old female C57BL/6 mice. Therapy was initiated in mice with tumor volume of 300 mm^3 (KLN205) or 500 mm^3 (E0771) and included control (Ctrl, vehicle, once per day), anti-PD1 (PD1, i.p. 10 mg/kg, every 3 days), glesatinib (gles, p.o. 60 mg/kg, q.d.), or anti-PD1 in combination with glesatinib at the indicated dose. Mice were treated for 2.5 weeks. **P* < 0.05, ***P* < 0.01 combo vs. glesatinib alone by *t* test.