

Tumor-intrinsic IRAK1 dependent inflammation signaling induces MDSC to establish immune evasion in FGFR1-driven hematological malignancies

Baohuan Cai, Yun Liu, Yating Chong, Gang Zhou, John K Cowell and Tianxiang Hu
Georgia Cancer Center, Augusta University, Augusta, GA 30912, USA

Background

Stem Cell leukemia/lymphoma syndrome (SCLL) presents as a myeloproliferative disease which can progress to acute myeloid leukemia and is associated with the coincident development of B-cell and T-cell lymphomas [1]. SCLL is driven by the constitutive activation of FGFR1 as a result of chromosome translocations with poor outcome. How the oncogenic FGFR1 signaling modulate the antitumor response and reshapes the immune landscape is completely unknown.

Objective

In this study, we use this model to investigate how FGFR1 fusion kinase drives an IRAK1[2] dependent inflammatory signaling in modulation of antitumor immunity.

Materials & Methods

CRISPR/Cas9 [3] was used to generate IRAK1 KO SCLL cells which were engrafted into syngeneic hosts. Development of the disease and changes in immune cell composition was monitored using flow cytometry. Bead-Based Immunoassay was used to identify the IRAK1 dependent inflammatory signals. IFNGR1 null mice and IRAK1 inhibitor pacritinib [4] were used for interruption of this immune evasion process.

Results

1. IARK1 expression and association with leukemia progression.

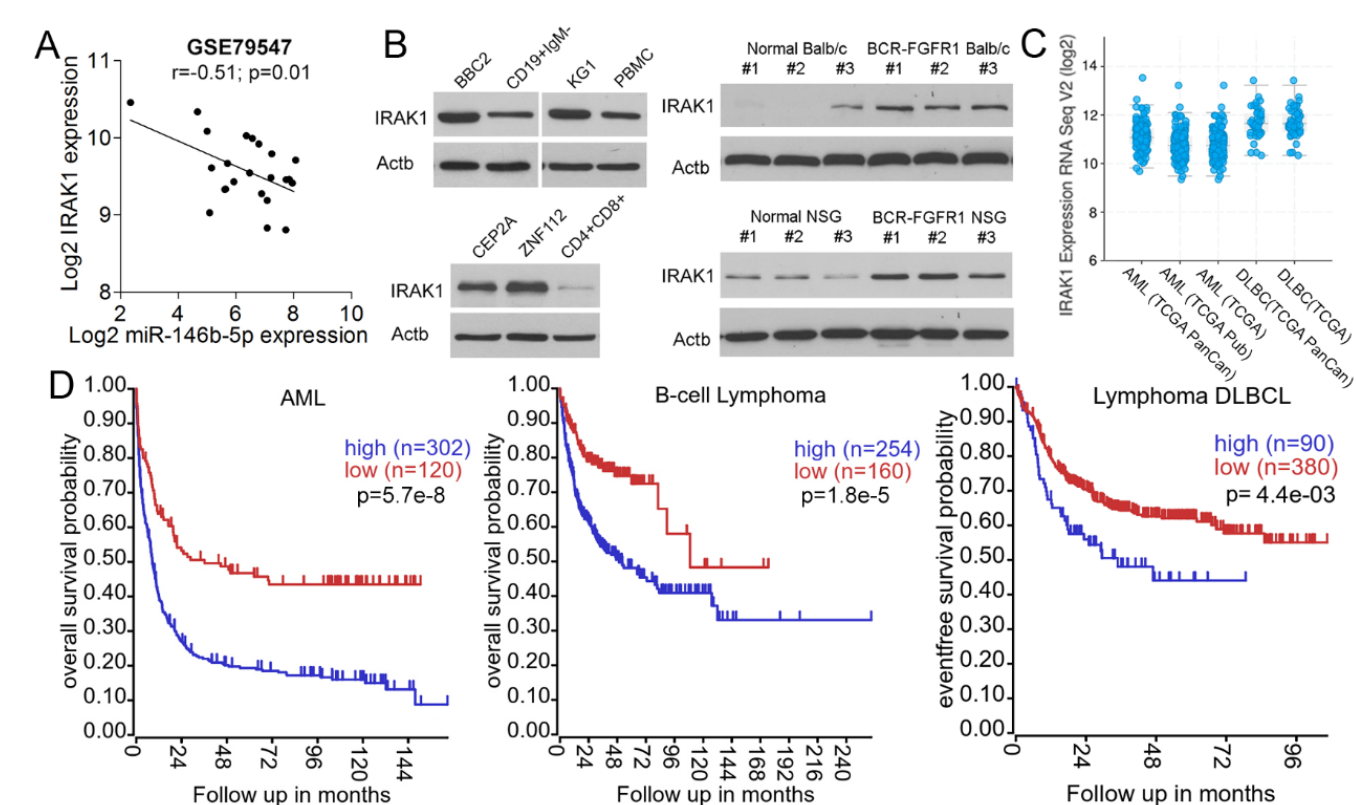


Fig 1. Overexpression of IRAK1 in blood cancer and correlation with disease prognosis. (A) An anti-correlation between IRAK1 and miR-146b-5p expression. (B) The consistent overexpression of IRAK1 in SCLL cell lines and primary tissues from mouse models. (C) The highly consistent overexpression of IRAK1 in different types of blood cancer, according to the Pan-Cancer Atlas data. (D) Patients with high IRAK1 expression show poor prognosis in AML, B-cell lymphoma and DLBCL.

2. IARK1 KO leads to immune mediated leukemia clearance.

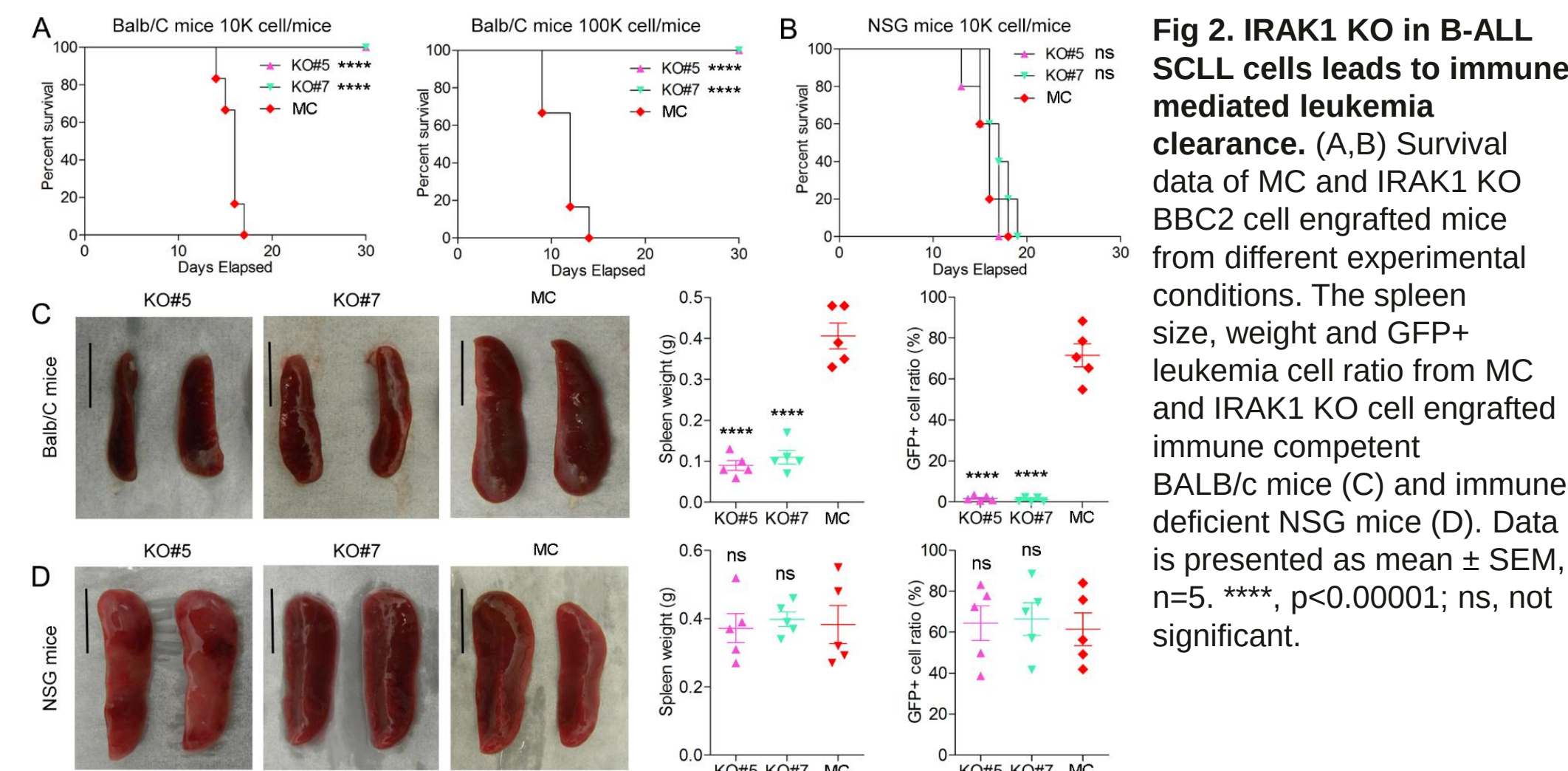


Fig 2. IRAK1 KO in B-ALL SCLL cells leads to immune mediated leukemia clearance. (A,B) Survival data of MC and IRAK1 KO BCC2 cell engrafted mice from different experimental conditions. The spleen size, weight and GFP+ leukemia cell ratio from MC and IRAK1 KO cell engrafted immune competent BALB/c mice (C) and immune deficient NSG mice (D). Data is presented as mean $\pm$ SEM, n=5, ****, p<0.00001; ns, not significant.

3. IARK1 is involved in MDSCs mediated T cell suppression.

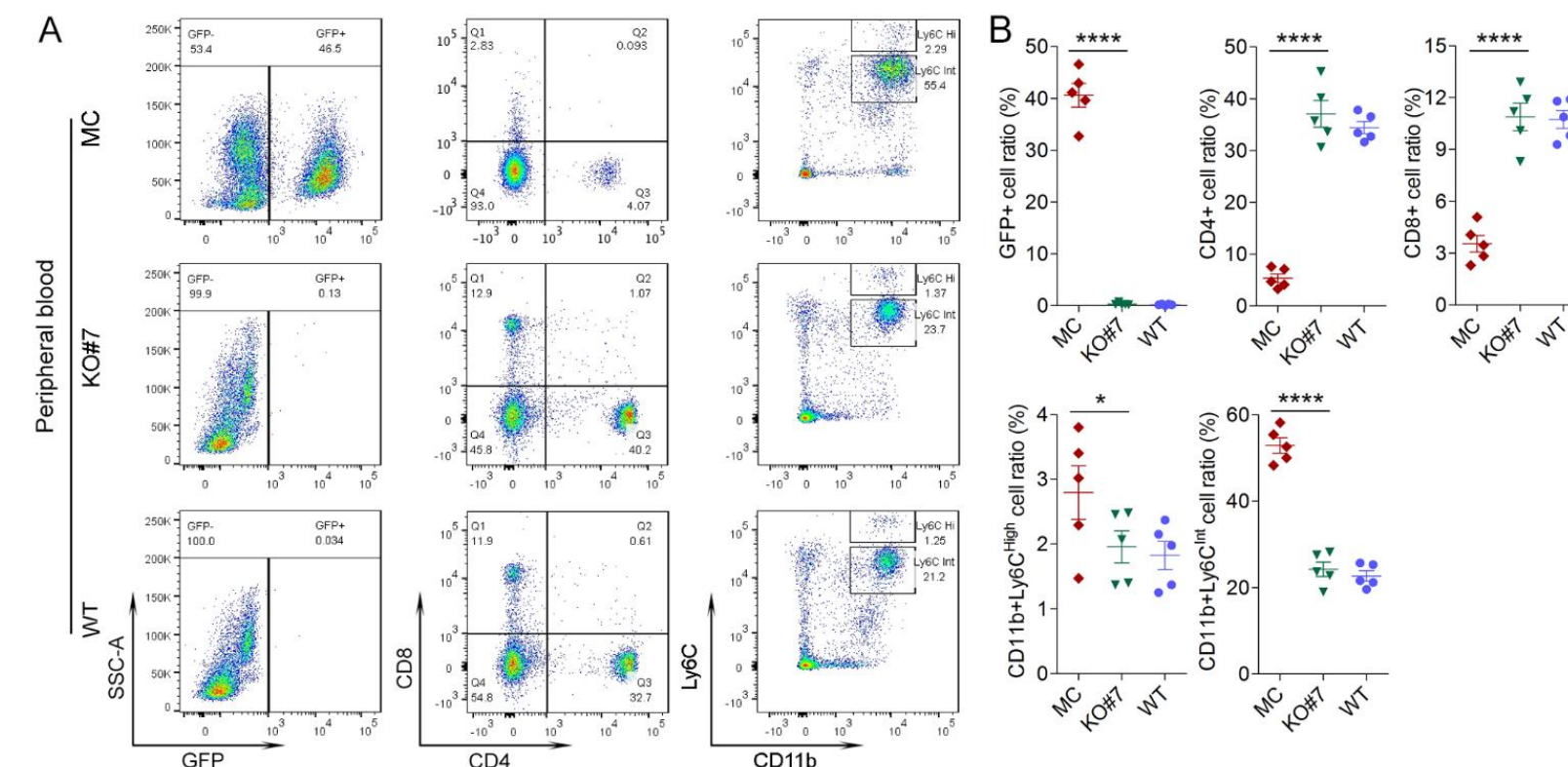


Fig 3. IRAK1 KO results in decreased MDSCs and increased T cells in B-ALL SCLL. Flow analysis of peripheral blood (A,B) from MC and IRAK1 KO cell engrafted mice at the time of sacrifice in presence of moribund syndrome. Statistical ratios for indicated immune cells in both samples shows significantly reduced CD11b+Ly6C^{high}/Ly6C^{int} MDSCs and increased CD4+ and CD8+ T cells in the IRAK1KO cells engrafted mice, compared to those engrafted with MC cells. The baseline ratios for each cell type are shown in the normal wild type (WT) mice. Data is presented as mean $\pm$ SEM, n=5, ****, p<0.00001.

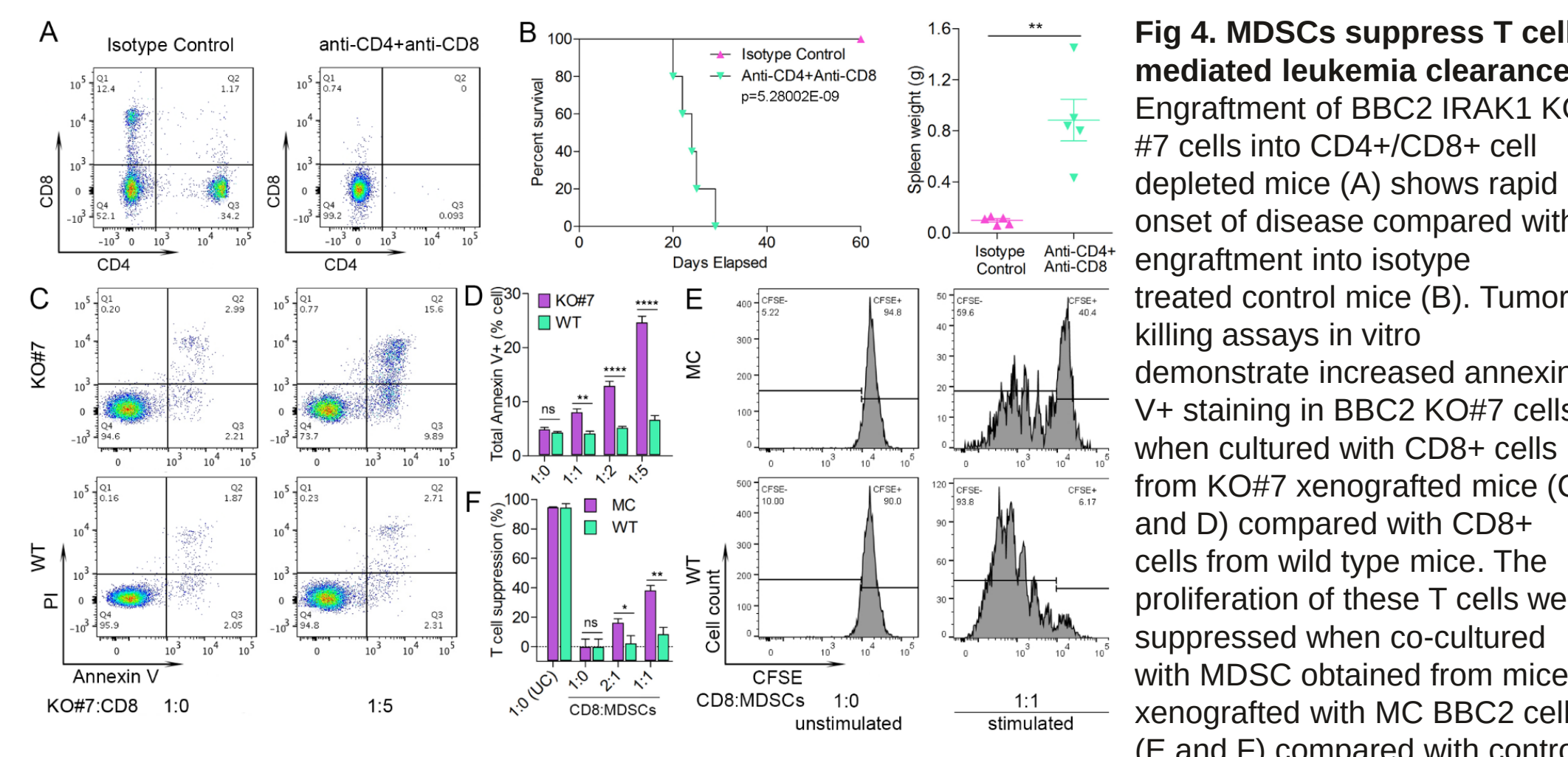
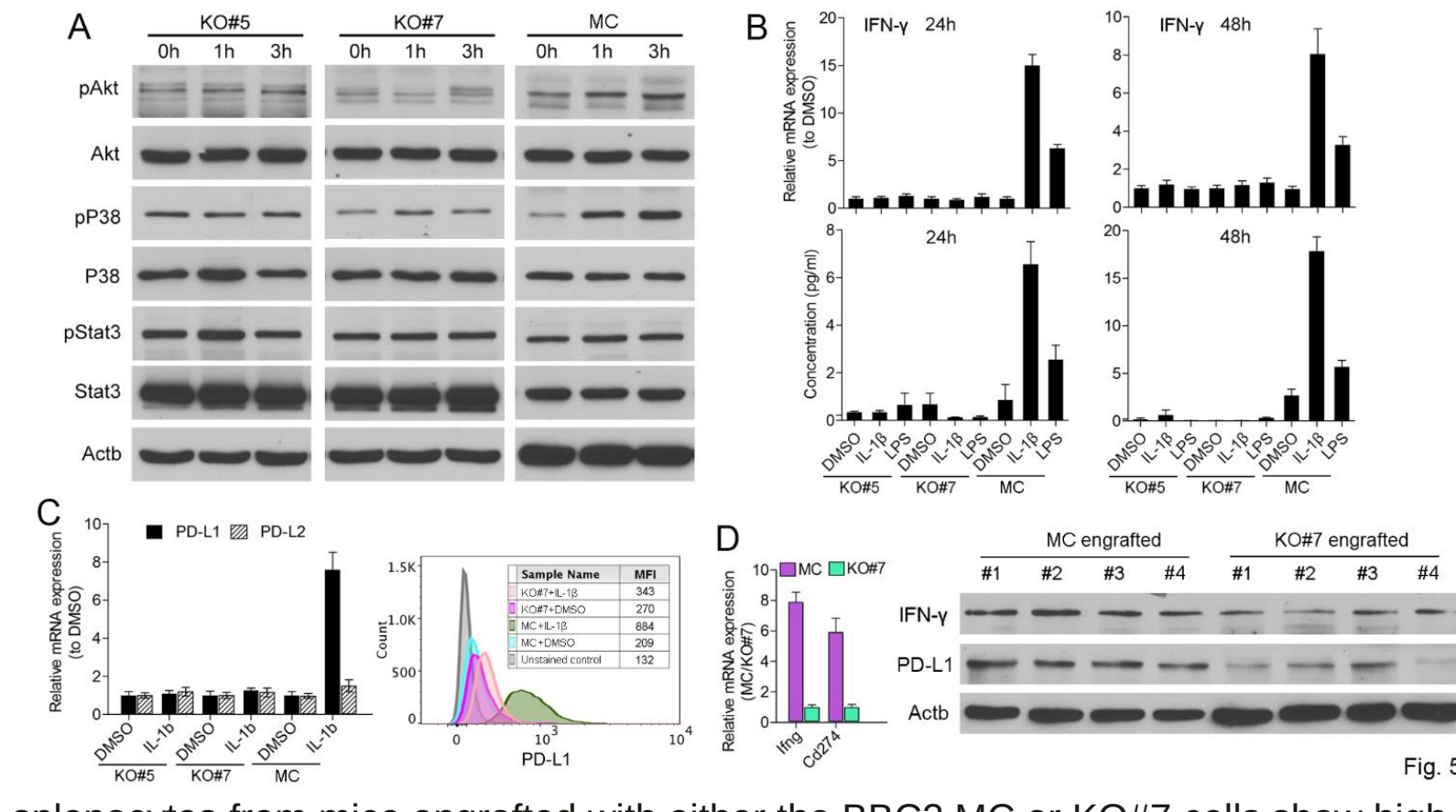


Fig 4. MDSCs suppress T cell mediated leukemia clearance. Engraftment of BCC2 IRAK1 KO #7 cells into CD4+/CD8+ cell depleted mice (A) shows rapid onset of disease compared with engraftment into isotype treated control mice (B). Tumor killing assays in vitro demonstrate increased annexin V+ staining in BCC2 KO#7 cells when cultured with CD8+ cells from KO#7 xenografted mice (C and D) compared with CD8+ cells from wild type mice. The proliferation of these T cells were suppressed when co-cultured with MDSC obtained from mice xenografted with MC BCC2 cells (E and F) compared with control.

4. IARK1 regulated INF-γ activates PD-L1 expression.



splenocytes from mice engrafted with either the BBC2 MC or KO#7 cells show high level expression of INF- γ and PD-L1 in the MC cells engrafted mice.

5. INF-γ signal contributes to MDSC induction during leukemia.

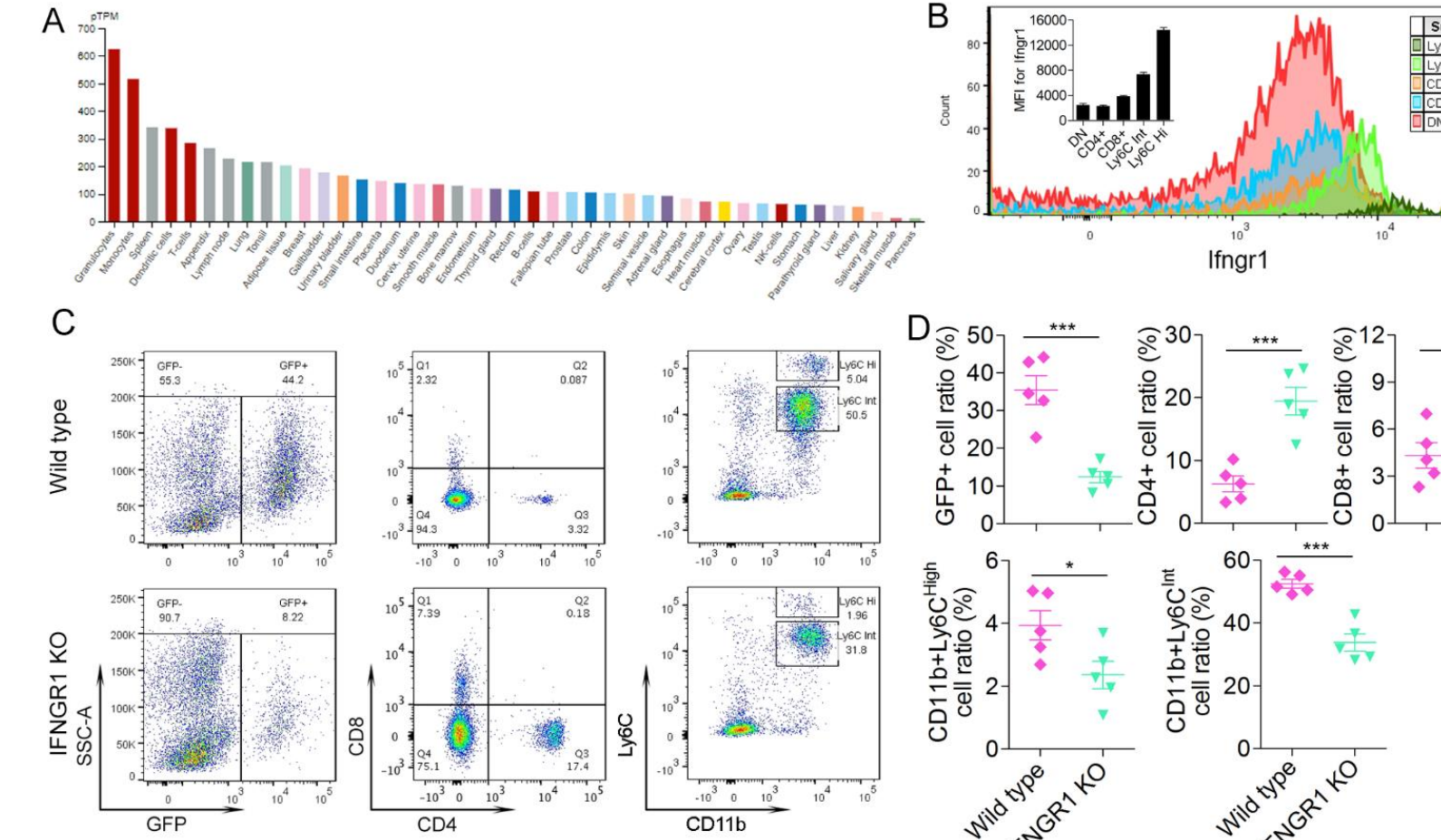


Fig 5. IRAK1 regulated INF- γ contributes to increased PD-L1 expression. Compared with the MC cells, IRAK1 KO clone cells show impaired Akt and P38 activation (A) and INF- γ production (B) when the cells were treated with 10ng/ml of IL-1 β for indicated time. (C) qRT-PCR detection of PD-L1/2 mRNA and flow detection of PD-L1 protein expression in MC and IRAK1KO cells treated for 24h, showing increased expression that correspond to levels of INF- γ . (D) qRT-PCR and western blot analysis of

in levels of Ly6C+/CD11b+ MDSC and an increase in CD4+/CD8+ cells compared with wild type mice engrafted with BCC2 cells (C) These changes are quantified in (D).

6. Targeting IRAK1 can attenuate leukemia progression.

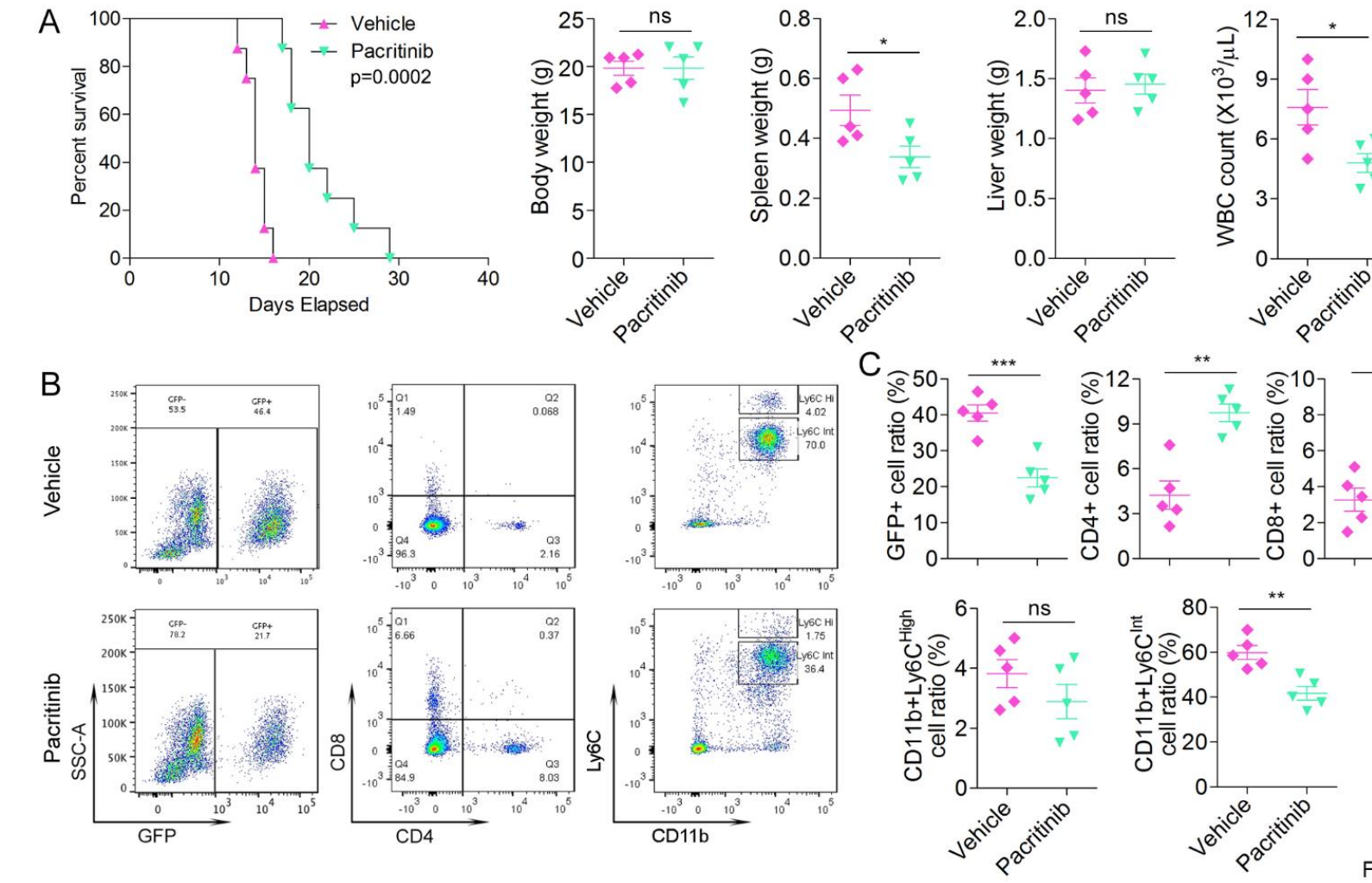


Fig 6. IFN- γ signal contributes to MDSC induction during the leukemogenesis. Relative expression levels (A) of the IFNGR1 in various cell types as reported in the Human Protein Atlas database. Flow cytometric analysis (B) of IFNGR1 confirms its predominant presence in both CD11b+Ly6C^{int} and CD11b+Ly6C^{hi} myeloid cells from wild type mice. Flow cytometry demonstrates reduced leukemic GFP+ cells in the spleens from Ifngr1 null mice, a reduction in CD4+/CD8+ T-cells compared with wild type mice engrafted with BCC2 cells (C) These changes are quantified in (D).

Fig. 7

Summary

Emerging evidence shows that tumor-intrinsic oncogenic pathways not only drive tumorigenesis, but also modulate the antitumor response by reshaping the immune landscape. Here we demonstrated that FGFR1 fusion kinase driven an IRAK1 dependent inflammatory signaling in leukemia immune evasion. In SCLL cells, IRAK1 knockout (KO) leads to loss of the typical rapid progression when xenografted into immunocompetent mice and the mice remained disease free. IRAK1 in this system has no effect on cell cycle progression or apoptosis and robust growth of the KO cells in immunodeficient mice suggested an effect on immune surveillance. Depletion of T-cells in immunocompetent mice restored leukemogenesis of the KO cells. Analysis of the immune cell profile in mice transplanted with the IRAK1 null cells shows that there is a reduction in levels of myeloid-derived suppressor cells (MDSCs) with a concomitant increase in CD4+/CD8+ T-cell levels. Immune profiling of a panel of secreted cytokines and chemokines showed that activation of INF- γ is specifically impaired in the KO cells. In vitro and in vivo expression assays and engraftment into IFNGR1 null mice, showed SCLL leukemia cells produced INF- γ not only promoting intrinsic PD-L1 expression, but also participating in the induction of MDSCs to establish immune evasion. Inhibition of IRAK1 using pacritinib suppresses leukemogenesis with impaired induction of MDSC cells and attenuated suppression of CD4+/CD8+ T-cells.

References

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Acknowledgements

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