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TITLE: TRANSCRIPTIONAL MODULATION OF TUMOR-ASSOCIATED MACROPHAGES TO FACILITATE PROSTATE CANCER IMMUNOTHERAPY

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14. ABSTRACT

Prostate cancer (PCa) contains abundant tumor-associated macrophages (TAMs), with increased TAM M2 polarization correlating with disease stage, emergence of castration-resistance, and worse prognosis. We hypothesize that targeting TAM transcription factors that mediate their M2 polarization, reprogramming them into the pro-inflammatory M1 state, will provide a novel approach to PCa therapy, and we seek to assess whether adoptive transfer monocytes lacking a key TAM transcription factor shows therapeutic utility, alone or with checkpoint inhibition. During the current reporting year we found that a murine PCa line grows slower in mice lacking NF-kB p50 compared with wild-type controls and in KLF4(f/f);Lys-Cre mice vs KLF4(f/f) controls, with increased TAM M1 polarization, particularly in p50-/- hosts, and with increased number and activation of tumor T cells, predominantly CD4 T cells in p50-/- vs wild-type and CD8 T cells in KLF4(f/f);Lys-Cre vs KLF4(f/f) tumor recipients. We also demonstrated that expansion of marrow progenitors in SCF/FL/TPO for 6 days, culture in M-CSF for 1 day, and adoptive transfer allows PCa tumor localization in preference to normal organs. And we find that anti-PD-1 is active in our tumor model. Thus, we have identified two transcription factors whose down-regulation in PCa TAMs contributes to tumor control and have begun to optimize a novel immunotherapy including adoptive transfer of gene modified myeloid cells.

15. SUBJECT TERMS

Prostate cancer, immunotherapy, tumor-associated macrophages, NF-kB p50, KLF4

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Transcriptional Modulation of Tumor-Associated Macrophages to Facilitate Prostate Cancer Immunotherapy

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1. INTRODUCTION

Prostate cancer (PCa) contains abundant tumor-associated macrophages (TAMs), with their increased content and percentage of M2 polarization correlating with increased disease stage, emergence of castration-resistant disease, and worse prognosis. We hypothesize that targeting TAM transcription factors that mediate their M2 polarization, reprogramming them into the pro-inflammatory M1 state, would provide a novel approach to prostate cancer therapy. This project first seeks to determine whether syngeneic murine PCa cells, unselected or castration resistant, grow more slowly in mice lacking NF-κB p50 (p50), KLF4, or PU.1 in their myeloid cells, correlated with increased TAM M1 polarization and increased activation of tumor-infiltrating lymphocytes. We then seek to assess whether adoptive transfer or murine monocytes lacking p50, KLF4, or PU.1 slows PCa growth, alone or in synergy with CSF1R small molecule tyrosine kinase inhibitor (TKI) or antibody (Ab) targeting or with anti-PD-1 or anti-CTLA4 T cell checkpoint inhibitory antibodies.

2. KEYWORDS

Prostate cancer, immunotherapy, tumor-associated macrophages, NF-κB p50, KLF4

3. ACCOMPLISHMENTS

Major goals of the project

Task 1: Assess prostate cancer growth in WT versus p50-/-, KLF4(f/f);Lys-Cre, and PU.1(kd/kd) B6 mice
- Subtask 1 - Obtain ACURO approval (mos 1-3), completed prior to 9/01/2016 start date
- Subtask 2 - Assess PCa growth in WT vs mutant recipients (mos 4-12), completed 6/01/17
- Subtask 3 - Assess castration-resistant growth in WT vs mutant recipients (mos 6-24), 75% complete
- Subtask 4 - Assess metastatic growth in WT vs mutant recipients (mos 6-24), 10% complete

Task 2: TAM polarization and T cell activation in WT vs p50-/-, KLF4(f/f);Lys-Cre, and PU.1(kd/kd) mice
- Subtask 1 - Obtain ACURO approval (mos 3-5), completed prior to 9/01/2016 start date
- Subtask 2 – Assess TAM polarization and T cell numbers and activation (mos 6-24), 90% complete.

Task 3: Assess the effect of adoptively transferred M2-defective M1 macrophages on prostate cancer
- Subtask 1 - Obtain ACURO approval (mos 2-5), completed prior to 9/01/2016 start date
- Subtask 2 - Determine effects of adoptively transferred monocytes (mos 6-24), completed 7/01/18
- Subtask 3 - Determine whether infused monocytes reach the tumor (mos 6-24), completed 7/01/18
- Subtask 4 - Determine how infused monocytes alter tumor TAMs and T cells (mos 6-24), 75% completed

Task 4: Assess synergy between CSF1R blockade and adoptive transfer of M2-defective M1 monocytes
- Subtask 1 - Obtain ACURO approval (mos 8-11), completed prior to 9/01/2016 start date
- Subtask 2 - Determine the effect of MCSFR TKI on prostate cancer growth (mos 12-36), 30% complete
- Subtask 3 - Determine synergy between MCSFR TKI and monocytes (mos 18-36), 0% complete
- Subtask 4 - Determine the effect of MCSFR TKI and monocytes on tumor TAMs/T cells (mos 18-36), 10%
- Subtask 5 - Determine synergy between MCSFR Ab and monocytes (mos 18-36), 10% complete
- Subtask 6 - Determine the effect of MCSFR Ab and monocytes on tumor TAMs/T cells (mos 18-36), 10%

Task 5: Assess synergy between checkpoint blockade and adoptive transfer of M2-defective M1 monocytes
- Subtask 1 - Obtain ACURO approval (mos 8-11), completed prior to 9/01/2016 start date
- Subtask 2 - Determine synergy between PD-1 Ab and monocytes on tumor growth (mos 12-36), 25%
- Subtask 3 - Determine synergy between CTLA4 Ab and monocytes on tumor growth (mos 12-36), 0%
- Subtask 4 - Determine synergy between PD-1/CTLA4 Ab and monocytes on tumor T cells (mos 12-36), 0%

Accomplishments under these goals

Task 1/Subtask 2 - Assess prostate cancer growth in WT versus p50, KLF4, and PU.1 mutant mice

Data showing significantly slowed Hi-Myc prostate cancer (PCa) growth in p50-/- versus WT and in KLF4(f/f);Lys-Cre vs KLF4(f/f) mice were provided in the Yr01 Progress Report. During Yr02, we were able to compare PCa tumor growth in a PU.1(kd/kd) male vs three WT males, finding no difference.

Task 1/Subtask 3 - Assess castration-resistant prostate cancer growth in WT versus mutant mice

Data showing slowed castration-resistant prostate cancer (CRPC) in KLF4(f/f);Lys-Cre compared with KLF4(f/f) mice and development of a CRPC cell line that can be maintained by SQ passage was described in the Yr01 Progress Report. During Yr02 we compared CRPC growth in WT vs p50-/- tumor recipients, finding a trend toward slower growth in the absence of host NF-κB p50.

Task 1/Subtask 4 - Assess metastatic growth in WT vs mutant recipients

We did not detect lung metastases in mice from whom primary, sq Hi-Myc PCa tumors were removed.

Task 2/Subtask 2 - Assess tumor TAM polarization and T cell numbers and activation in WT vs mutant hosts

Data showing reduced M2 and increased M1 tumor-associated macrophage (TAM) polarization in $p50^{-/-}$ compared with WT hosts and in KLF4(f/f);Lys-Cre vs KLF4(f/f) PCa hosts was presented in the Yr01 Progress Report. In addition, data showing increased total and activated tumor CD4 T cells in $p50^{-/-}$ hosts and increased total and activated tumor CD8 T cells in KLF4(f/f);Lys-Cre hosts was provided. During Yr02, we demonstrated that CD8 T cell depletion via CD8 Ab injections prior to tumor inoculation obviated the PCa growth difference in KLF4(f/f);Lys-Cre vs KLF4(f/f) hosts. Global gene expression analysis of $CD11b^{+}$ myeloid cells from PCa tumor growing in KLF4(f/f);Lys-Cre vs KLF4(f/f) hosts was also described in the Yr01 Progress Report. During Yr02, we completed Ingenuity Pathway Analysis of these data, finding that absence of KLF4 in PCa tumor myeloid cells leads to activation of pro-inflammatory Atherosclerosis Signaling as the top pathway activated.

Task 3/Subtask 2 - Determine effects of adoptively transferred monocytes

Our Yr01 and Yr02 progress reports described our method for generating immature myeloid cells from p50-/- or wild-type, lineage-negative murine bone marrow cells, designated p50-IMC or WT-IMC respectively. The Yr02 report also described the flow cytometry characteristics of these IMC, showing that they include both monocytic ($CD115^{hi}$) and dendritic ($Flt3^{hi}CD11c^{+}$) precursors.

During Yr03, we extended our data showing that 5FU followed by p50-IMC, but not 5FU alone, p50-IMC alone, or 5FU followed by WT-IMC, slows the growth of prostate tumors. Hi-Myc PCa was inoculated into the flanks of syngeneic B6 male mice. Thirteen days later, when tumors were just palpable, mice received 5FU, followed by three intravenous injections of 1E7 WT-IMC or p50-IMC on days 18, 21, and 25, as diagrammed (Fig. 1a, top). Additional groups of mice received 5FU alone, p50-IMC alone, or neither. Tumor volumes measured at individual time points for each mouse in these five experimental groups are shown (Fig. 1a, bottom). Measured tumor volumes were fit to an exponential model of tumor growth (Fig. 1b). Between days 19 and 35, expected mean tumor volumes for the 5FU/p50-IMC group were significantly lower than those for both the 5FU/WT-IMC and 5FU alone groups, with $p=0.0001$ on day 29. We also compared the measured tumor volumes on day 28 or 29 between these groups. At this time, the mean volume of tumors in the 5FU/p50-IMC group was 3-fold lower than that for the 5FU group ($p<0.001$), and 4-fold lower than that of the 5FU/WT-IMC group ($p<0.0001$). Of note, this analysis underestimates the true differences in tumor sizes because 4 tumors in the 5FU group and 4 tumors in the 5FU/WT-IMC group had already reached 2 cm and required euthanasia prior to day 28 (Fig. 1c, top). These data indicate that adoptive transfer of p50-IMC, but not WT-IMC, slows PCa tumor growth when administered after a dose of 5FU. Comparison of modeled tumor growth between the untreated and p50-IMC alone groups revealed no significant difference in expected mean tumor volumes between day 15 and day 38, whereas 5FU/p50-IMC lead to significantly smaller expected mean tumor volumes on each of these days. Comparison of actual tumor volumes on day 25 confirms that there exists no difference between no treatment and p50-IMC alone; conversely, the mean tumor volume for the 5FU/p50-IMC group is 9-fold lower than that of the p50-IMC alone group (Fig. 1c, bottom). Thus, p50-IMC does not slow PCa tumor growth without preceding 5FU.

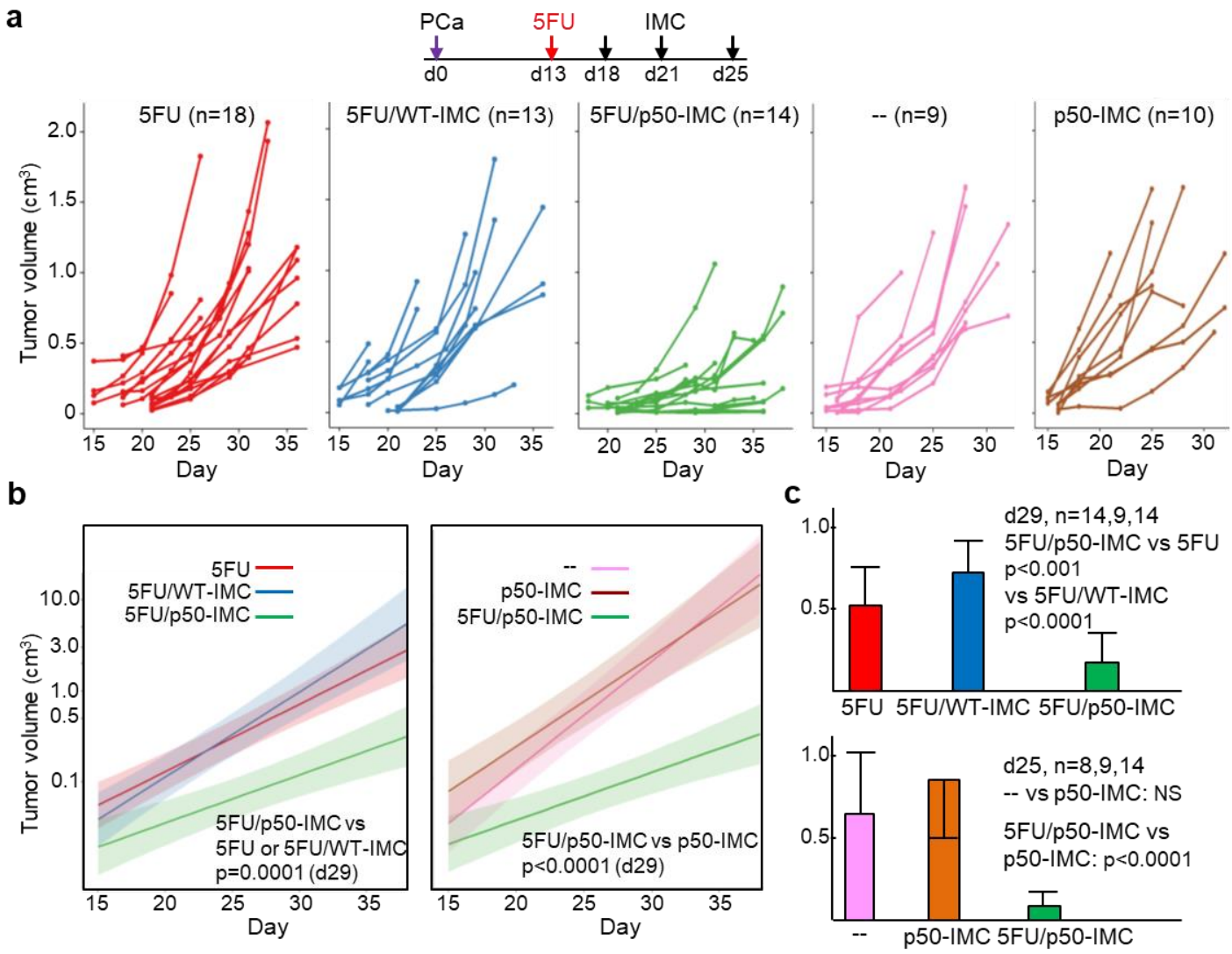

Fig. 1 5FU followed by p50-IMC slows PCa tumor growth. (**a**) Mice inoculated SQ with Hi-Myc PCa on day 0 received 5FU on day 13 and 1E7 WT-IMC or p50-IMC on days 18, 21, and 25, as diagrammed. Additional groups of mice received 5FU alone, no treatment, or p50-IMC alone. Individual tumor volumes as measured every two to four days are shown. (**b**) These data were fit to an exponential model, with semi-log plots and comparison of expected mean tumor volumes between the 5FU/p50-IMC and 5FU/WT-IMC, 5FU alone, or p50-IMC alone groups on day 29 shown. (**c**) Mean tumor volumes and SEs as measured on day 29 (or day 28) for 5FU, 5FU/WT-IMC, and 5FU/p50-IMC mice (top) and on day 25 for no treatment (--), p50-IMC, and 5FU/p50-IMC are shown along with the number of mice with measurable tumors in each group on those days.

Since p50-IMC obtained by culture for six days with TPO/SCF/FL and then for one day in M-CSF may include potential DC precursors, we examined whether culture with FL and low-dose GM-CSF rather than M-CSF could augment anti-tumor efficacy, as this cytokine combination favors DC formation from cultured marrow cells. PCa tumor growth in individual mice treated with 5FU on day 13 followed by p50-IMC(GM+FL) on days 18, 21, and 25 is shown (Fig. 2a). Tumor volumes for these groups were fit to an exponential model (Fig. 2b), and measured tumor volumes on day 25 were compared (Fig. 2c), revealing that p50-IMC generated using GM+FL are equally effective, but not more effective, than those generated using M-CSF.

We also examined whether a lower dose of p50-IMC per intravenous injection would be as effective as 1E7 IMC per dose. Cohorts of mice inoculated with PCa received a dose of 5FU on day 13 followed by three infusions of p50-IMC generated using M-CSF at either 4E6 or 1E6 cells per dose. Tumor growth in individual mice for these two groups is shown (Fig. 2d). The 4E6 dose appeared more effective than the 1E6 dose. Analysis of the

5FU/p50-IMC(4E6) data in comparison with 5FU/p50-IMC(1E7) and 5FU data from Fig. 1 using the exponential model reveals that 5FU followed by p50-IMC(4E6) is not significantly better than 5FU alone between day 15 and day 40 (Fig. 2e), although a trend towards increased efficacy may exist, as indicated also by comparing tumor volumes on day 23, with p=0.12 (Fig. 2f). These data also demonstrate that 5FU/p50-IMC(1E7) is more effective than 5FU/p50-IMC(4E6).

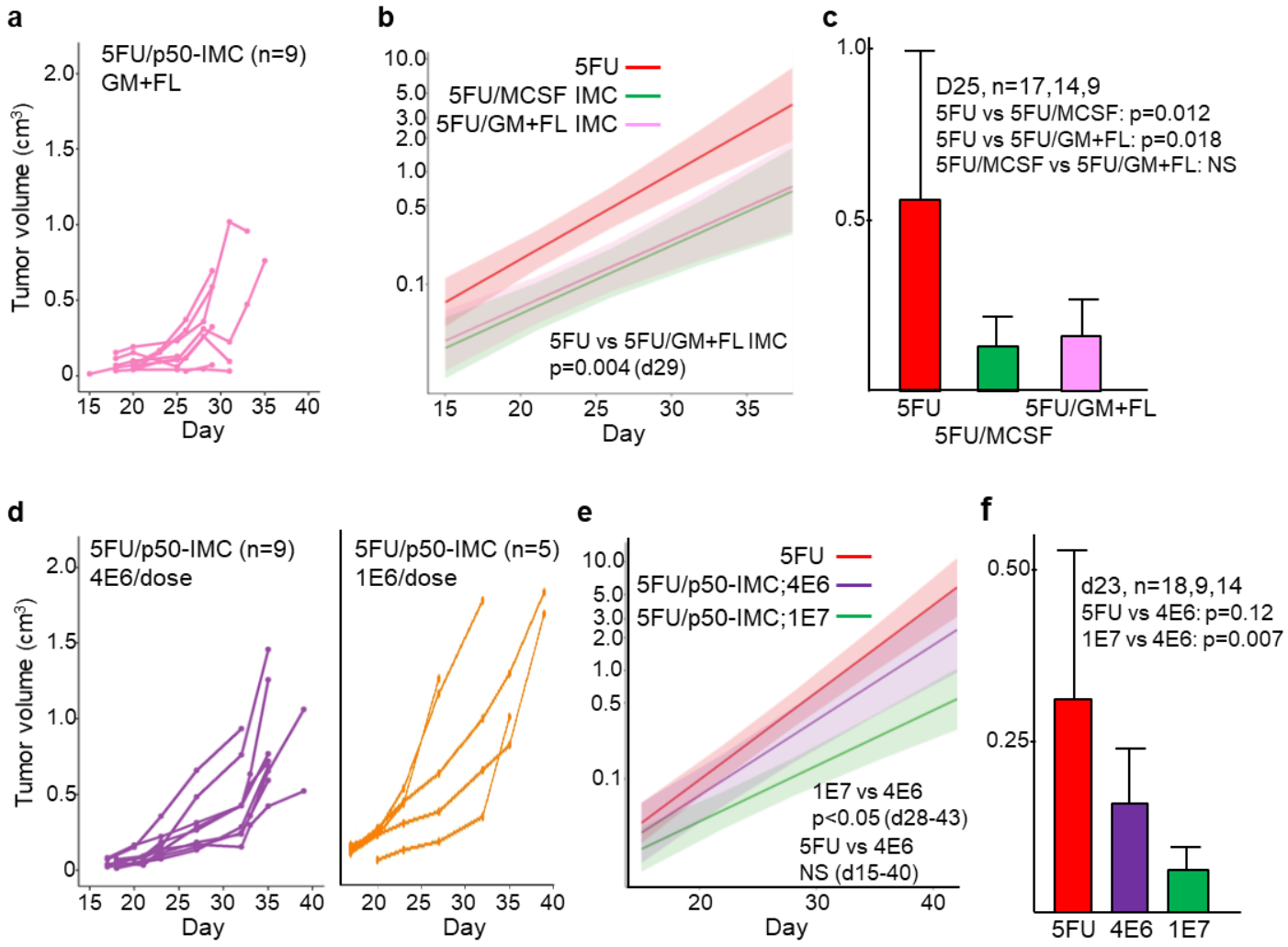

Fig. 2 Efficacy of p50-IMC generated using FL and low-dose GM-CSF and p50-IMC dose-response. (**a**) Individual tumor volumes as measured every two to four days are shown for treatment with 5FU alone, 5FU/p50-IMC(M-CSF), or 5FU/p50-IMC(GM+FL). **b**) These data were fit to an exponential model, with semi-log plots and comparison of expected mean tumor volumes between the 5FU and 5FU/p50-IMC(GM+FL) groups on day 29 shown. (**c**) Mean tumor volumes and SEs as measured on day 25 (or day 26) are shown. (**d**) PCa tumor growth data in response to 5FU followed by three doses of 4E6 or 1E6 p50-IMC are shown. (**e**) These data and data from Fig. 1 for 5FU alone were fit to an exponential model, with semi-log plots and comparison of expected mean tumor volumes between the 5FU and 5FU/p50-IMC(4E6) groups shown for days 15-40. (**f**) Tumor volumes as measured on day 23 (or day 22) for these two groups are also compared.

Task 3/Subtask 3 - Determine whether infused monocytes reach prostate cancer tumors

Our Yr02 progress report described tumor localization of WT-IMC generated from CMV-Luc mice, as assessed by IVIS imaging. Our Yr02 report also provided initial data on the fate of CD45.2$^+$ p50-IMC injected into CD45.1$^+$ prostate cancer tumor-bearing hosts. During Yr03 we have made substantial additional progress characterizing the fate of transferred CD45.2$^+$ p50-IMC vs WT-IMC in both tumor and lymph nodes.

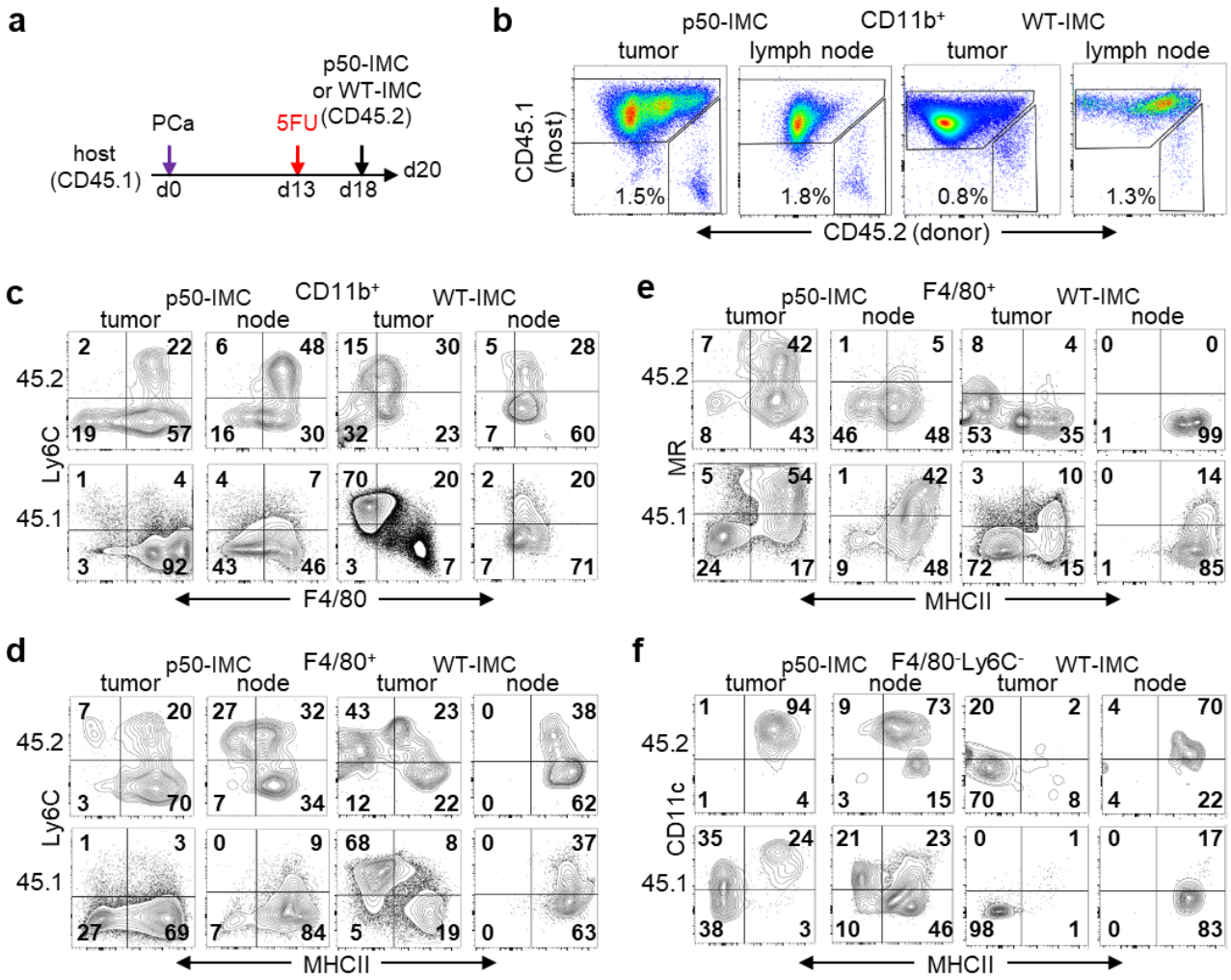

Fig. 3. p50-IMC generate increased PCa tumor and lymph node myeloid cells and dendritic cells compared with WT-IMC. (**a**) CD45.1$^+$ mice inoculated with PCa on day 0 received 5FU on day 13 and 1E7 p50-IMC or WT-IMC derived from CD45.2$^+$ mice on day 18, followed by tumor and inguinal lymph node mononuclear cell isolation and analysis on day 20, as diagrammed. Data acquired from 2E4 CD11b$^+$ cells per mouse from five mice in a single experiment were pooled for analysis. (**b**) CD11b$^+$ myeloid cells were analyzed for CD45.2$^+$ (p50-IMC donor-derived) and CD45.1$^+$ (host) cells by FC. (**c**) IMC and host myeloid cells were analyzed for Ly6C and F4/80. (**d**) F4/80$^+$ myeloid cells within the CD45.2$^+$ and CD45.1$^+$ populations were analyzed for Ly6C and MHCII. (**e**) F4/80$^+$ myeloid cells within the CD45.2$^+$ and CD45.1$^+$ populations were analyzed for MR and MHCII. (**f**) F4/80$^-$Ly6C$^-$ myeloid cells within the CD45.2$^+$ and CD45.1$^+$ populations were analyzed for CD11c and MHCII.

B6 mice inoculated with Hi-Myc PCa received 5FU on day 13 followed by 1E7 CD45.2$^+$ p50-IMC or WT-IMC on day 18, with isolation of tumor and inguinal draining lymph nodes two days later, as diagrammed (Fig. 3a). As IMC contribution to total tumor or lymph node CD11b$^+$ myeloid cells was low, flow data acquired individually from four WT-IMC and five p50-IMC mice were pooled to generate the flow plots and populations frequencies shown. WT-IMC accounted for 0.8% of tumor and 1.3% of lymph node CD11b$^+$ myeloid cells, whereas p50-IMC comprised a higher proportion at 1.5% and 1.8%, respectively. (Fig. 3b). When data from the four WT-IMC and five p50-IMC mice are compared, the increased frequency of p50-IMC within tumor myeloid cells shows a trend towards significance (p=0.12), and the increased contribution of p50-IMC to nodal myeloid cells is significant (p=0.007). Consistent with data obtained with Luc-IMC, p50-IMC also localized to spleen and marrow, where they formed 7.7% and 11% of CD11b$^+$ myeloid cells, respectively (not shown). Only 1.3% of tumor and 1.5% of nodal myeloid cells derived from p50-IMC were Ly6G$^+$ neutrophils, while 2.2% of tumor and 5.8% of lymph node myeloid cells derived from WT-IMC were Ly6G$^+$ (not shown). The F4/80 macrophage

marker was expressed on 53% of tumor myeloid cells derived from WT-IMC and 79% of those derived from p50-IMC (p=0.001); in lymph nodes, however, the frequencies of IMC-derived F4/80$^+$ macrophages (88% vs 78%) were not significantly different (Fig. 3c). A substantial fraction of F4/80$^+$ macrophages also expressed Ly6C, which is present on inflammatory monocytes and on both immune-activating and suppressive macrophages. As activated macrophages can co-express Ly6C with MHCII, we analyzed both markers (Fig. 3d). WT-IMC-derived macrophages co-expressed Ly6C and MHCII to a similar extent as p50-IMC-derived macrophages (23% vs 20% in tumor and 38% vs 32% in lymph nodes), both not significant (NS). We also evaluated F4/80$^+$ macrophages for mannose receptor (MR), a marker of tumor-promoting M2-polarized macrophages (Fig. 3e). In p50-IMC, macrophage MR was increased in tumor (49% vs 12%, p=0.001) and decreased in lymph node (6% vs 13%, NS) compared to WT-IMC. In addition to F4/80$^+$ macrophages, IMC-derived myeloid cells included a population of F4/80$^-$Ly6C$^-$ cells; in tumor, their frequency was higher in WT-IMC than p50-IMC (32% vs 19%), whereas in lymph node their frequency was lower in WT-IMC than p50-IMC (7% vs 16%). F4/80$^-$Ly6C$^-$ cells were further evaluated for CD11c and MHCII, markers co-expressed on activated DCs (Fig.3f). Strikingly, 94% of p50-IMC-derived tumor F4/80$^-$Ly6C$^-$ cells co-expressed the CD11c and MHCII DC markers, compared with only 2% of WT-IMC-derived cells (p<0.001). Although the frequency of p50-IMC-derived and WT-IMC-derived CD11c$^+$MHCII$^+$ DCs were similar in lymph node, the 2.3-fold increased frequency of the parent F4/80$^-$Ly6C$^-$ population indicates an overall increase in DCs derived from p50-IMC. The greater frequency of CD45.2$^+$ p50-IMC in the tumor and lymph node and the increased ability of p50-IMC to generate CD11c$^+$MHCII$^+$ cDCs in tumor and lymph node may partly account for the enhanced efficacy of p50-IMC compared with WT-IMC.

Task 3/Subtask 4 - Determine how infused monocytes alter tumor TAMs and T cells

The data presented in Fig. 3, largely acquired during Yr03, describe not only the fate of infused CD45.2$^+$ IMC but also the effect on host CD45.1$^+$ IMC, as described in the preceding section. Our Yr02 progress report provided data demonstrating that 5FU followed by three infusions of p50-IMC increased the number of tumor CD8 T cells 5-fold and the number of tumor CD8 T cells that express that express IFNγ (a T cell activation marker) in response to PMA/ionomycin 2-fold, compared to WT-IMC. During Yr03 we demonstrated that antibody-mediated CD8 T cell depletion obviates the slowed tumor growth seen with 5FU followed by three doses of p50-IMC, demonstrated that tumor response occurs, at least in large part, via CD8 T cell activation (Fig. 4).

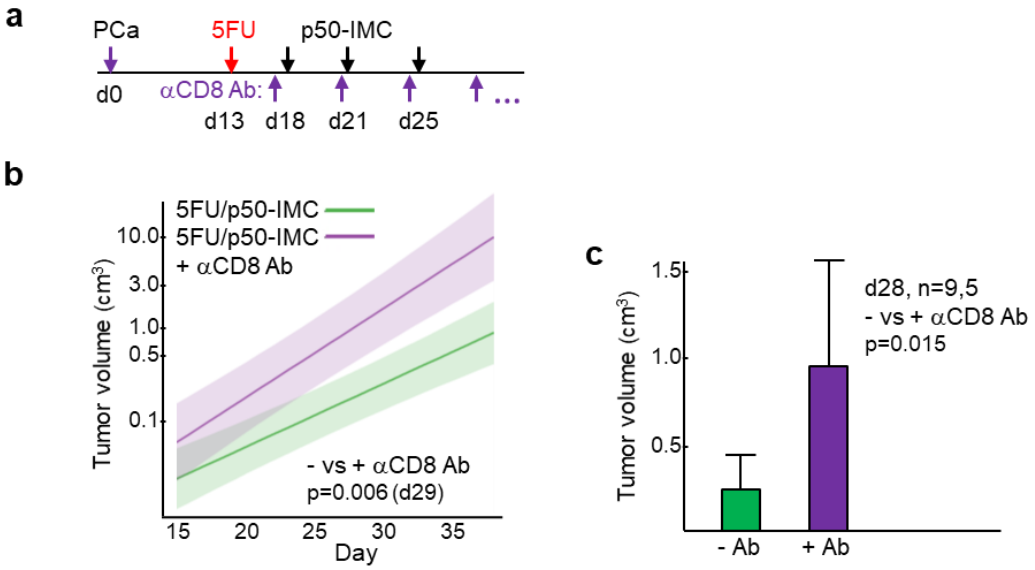

Fig. 4 (**a**) Mice inoculated with Hi-Myc PCa on day 0 received 5FU on day 13 and 1E7 WT-IMC or p50-IMC on days 18, 21, and 25 with or without CD8 antibody treatment twice weekly starting on day 17, as diagrammed. Individual tumor volumes as measured every two to four days are shown. (**b**) These data were fit to an exponential model, with semi-log plots and comparison of expected mean tumor volumes between the 5FU/p50-IMC and 5FU/p50-IMC plus anti-CD8 antibody (αCD8 Ab) groups on day 29 shown. (**c**) Tumor volumes as measured on day 18 for these two groups are also compared.

Task 4 - Assess synergy between CSF1R blockade and adoptive transfer of M2-defective M1 monocytes

The Yr01 Progress Report presented data indicating that MCSFR Ab could be detected in serum 2 days but not 6 days after three i.p. injections, based on the ability serum to stain exogenous MCSFR expressed on the surface of Ba/F3 cells. We concluded that IMC infusions will need to be delayed 5-6 days post-MCSFR Ab. During Yr02, we evaluated the effect of MCSFR Ab and of PLX3397, an MCSFR tyrosine kinase inhibitor (TKI), on PCa tumor myeloid cell numbers and tumor growth. MCSFR Ab reduced total and F4/80^{+} tumor myeloid cells ~2-fold, whereas had little effect. MCSFR Ab did not affect tumor growth, with a trend towards smaller tumors with PLX3397. We have not yet assessed the effect of combining CSF1R blockade with p50-IMC.

Task 5 - Assess synergy between checkpoint blockade and adoptive transfer of M2-defective M1 monocytes

The Yr01 Progress Report presented data showing that absence of host myeloid KLF4 or three dose of anti-PD-1 Ab (250 μg/dose) markedly slows PCa tumor growth, with the combination leading to a further mild reduction in tumor volume. In an effort to better demonstrate synergy, during Yr02 we repeated this experiment using one 250 μg dose of anti-PD-1. There was a trend toward slowed tumor growth when anti-PD-1 Ab was combined with absence of myeloid KLF4. During Yr02 we also evaluated synergy between 5FU/p50-IMC and three doses of anti-PD-1 Ab, finding no evidence for cooperation. During Yr03 we repeated this experiment and again found no evident synergy, in part due to the high potency of anti-PD-1 alone in this tumor model.

Opportunities for training and professional development

This proposal facilitated laboratory-based training and professional development in the fields of prostate cancer research and immunotherapy for two post-doctoral fellows, David Barakat, Ph.D. (Yr01 and Yr02) and Rahul Suresh, Ph.D. (Yr01, Yr02, and Yr03). In addition to conducting the above experiments, Drs. Barakat and Suresh attended and presented at weekly laboratory meeting held by Drs. Friedman and Pienta, and attended numerous scientific seminars at the Johns Hopkins Comprehensive Cancer Center, including Oncology Grand Rounds, Translational Research Conference, and Journal Club. Dr. Suresh presented his findings regarding the efficacy of p50-IMC against PCa as a poster presentation at the Johns Hopkins Prostate Cancer Research Day symposium (12/07/2017) and at the Johns Hopkins Cancer Center Fellow Research Day symposium (6/14/2018 and 6/18/2019). In addition, Dr. Suresh will present his findings at the American Association of Cancer Research Special Conference on Tumor Immunology and Immunotherapy (11/18/2019).

Dissemination of research results

Nothing to Report.

Plans during the next reporting period

Nothing to Report.

4. IMPACT

Impact on the development of the principal discipline(s) of the project

Prostate cancers include normal white blood cells called macrophages that contribute to tumor growth. These tumor-associated macrophages (TAMs) suppress the immune system's ability to fight prostate cancer. This proposal seeks to alter prostate cancer TAMs so that instead of helping the cancer grow they now help the immune system fight the cancer. Like all cells, TAMs contain genes within their DNA that govern their function. Our results indicate that removal of the KLF4 gene or the p50 gene from prostate cancer TAMs slows prostate cancer growth by activating the immune system, in particular CD8 T cells, to fight the cancer. In addition, we find that intravenous infusion of white blood cells lacking p50 into normal mice slows the growth of prostate cancer. These findings have implications for the treatment of prostate cancer, as we can now pursue a therapy in which we target KLF4 or p50 in TAMs, either within the tumor or in myeloid cells prior to their infusion.

Impact on other disciplines

In addition to prostate cancer, many other cancer contain TAMs that contribute to tumor growth, including brain, pancreatic, and breast cancers. Our findings are therefore also relevant to these and other cancers as targeting TAM KLF4 or p50 may also be effective for these cancers, alone or in combination with other therapies.

Impact on technology transfer

Our invention of p50-IMC and demonstration of efficacy against PCa when given after 5FU, has the potential to lead to transfer to industry and ultimately to clinical practice in patients with prostate cancer and other malignancies.

Impact on society beyond science and technology

Nothing to Report.

5. CHANGES/PROBLEMS

Changes in approach

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

6. PRODUCTS

Journal publications

The following peer-reviewed research manuscript was published during Yr02:

Authors Barakat DJ, Suresh R, Barberi T, Pienta K, Simons BW, Friedman AD.
Title Absence of myeloid Klf4 reduces prostate cancer growth with pro-atherosclerotic activation of tumor myeloid cells and infiltration of CD8 T cells
Journal PLoS One
Volume:year 13:2018
Page numbers e0191188 (16 pages)
Status of publication published
Acknowledgment of federal support Yes

The following peer-reviewed research manuscript was submitted during Yr03 (currently under review):

Authors Suresh S, Barakat DJ, Barberi T, Zheng L, Jaffee EM, Pienta KJ, Friedman AD.
Title NF-κB p50-deficient immature myeloid cell (p50-IMC) adoptive transfer slows the growth of murine prostate and pancreatic ductal carcinoma
Journal pending review and acceptance
Volume:year pending review and publication
Page numbers pending review and publication
Status of publication submitted
Acknowledgment of federal support Yes

Books or other non-periodical, one time publications
Nothing to Report.

Other publications, conference papers, and presentations
The following poster was presented at the 12th Annual Johns Hopkins Prostate Research Day on Dec. 7, 2017:

Suresh R, Barakat DJ, Gilmore GL, Simons B, Pienta KJ, Friedman AD. "Adoptive Transfer of Reprogrammed Myeloid Cells As a Component of Prostate Tumor Immunotherapy"

The following poster was presented at the Johns Hopkins University School of Medicine 2018 Sidney Kimmel Comprehensive Cancer Center Fellow Research Day on June 14, 2018:

Suresh R, Barakat DJ, Gilmore GL, Simons B, Pienta KJ, Friedman AD. "Adoptive Transfer of NF-κB Deficient Myeloid Cells As a Component of Prostate Cancer Immunotherapy"

The following poster was presented at the Johns Hopkins University School of Medicine 2019 Sidney Kimmel Comprehensive Cancer Center Fellow Research Day on June 18, 2019:

Suresh S, Barakat DJ, Barberi T, Zheng L, Jaffee EM, Pienta KJ, Friedman AD. "NF-κB p50-deficient immature myeloid cell (p50-IMC) adoptive transfer slows the growth of murine prostate and pancreatic ductal carcinoma"

The following poster was presented at the Annual Meeting of the Society for Immunotherapy of Cancer on November 8, 2019:

Suresh S, Barakat DJ, Barberi T, Zheng L, Jaffee EM, Pienta KJ, Friedman AD. "NF-κB p50-deficient immature myeloid cell (p50-IMC) adoptive transfer slows the growth of murine prostate and pancreatic ductal carcinoma"

The following poster will be presented at the American Association of Cancer Research Special Conference on Tumor Immunology and Immunotherapy on November 18, 2019:

Suresh S, Barakat DJ, Barberi T, Zheng L, Jaffee EM, Pienta KJ, Friedman AD. "NF-κB p50-deficient immature myeloid cell (p50-IMC) adoptive transfer slows the growth of murine prostate and pancreatic ductal carcinoma"

Websites or other internet sites
Nothing to Report.

Technologies or techniques
Nothing to Report.

Inventions and Patent Applications
An Invention Disclosure, "Adoptive Cell Transfer of NF-κB p50 Deficient Immature Myeloid Cells (ACT of p50-IMC)" was made to iEDISON by the Johns Hopkins Technology Ventures (JHTV) office ~4 weeks after initial disclosure to JHTV. The iEDISON EIR# is 4134401-18-0009.

A provisional patent application, #62677815, was made on May, 30, 2018 to the United States Patent and Trademark Office entitled "NF-κB p50 Deficient Immature Myeloid Cells and Their Use in Treatment of Cancer."

Other products
Data We find that prostate cancer grows significantly slower in mice lacking NF-κB p50 or macrophage KLF4, or in mice with prostate cancer that are given 5FU followed by p50-IMC, associated with increased tumor macrophage and T cell activation. These findings provide a meaningful contribution towards understanding gene regulatory mechanisms that allow TAMs to contribute to prostate cancer growth and towards developing of novel therapy approaches designed to modify tumor TAMs.

Research materials We have developed a C57BL/6 murine cell line model of castration-resistant prostate cancer (CRPC) from castration-sensitive B6 Hi-Myc PCa.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Individuals who worked on the project during Yr03 (Yr03 effort round up to nearest whole month)

Name: Alan D. Friedman
Project Role: Principal Investigator
Person months worked: 1
Contribution to Project: no change
Funding Support: this award

Name: Kenneth J. Pienta
Project Role: Co-investigator
Person months worked: 1
Contribution to Project: no change
Funding Support: this award

Name: Rahul Suresh
Project Role: Post-doctoral Fellow
Person months worked: 12
Contribution to Project: no change
Funding Support: this award

Individuals who worked on the project during Yr01-03 (Yr01-03 effort round up to nearest whole month)

Name: Alan D. Friedman
Project Role: Principal Investigator
Person months worked: 3
Contribution to Project: no change
Funding Support: this award

Name: Kenneth J. Pienta
Project Role: Co-investigator
Person months worked: 3
Contribution to Project: no change
Funding Support: this award

Name: David J. Barakat
Project Role: Post-doctoral Fellow
Person months worked: 24
Contribution to Project: no change
Funding Support: this award

Name: Rahul Suresh
Project Role: Post-doctoral Fellow
Person months worked: 28
Contribution to Project: no change
Funding Support: this award

Change in the active other support of the PI and senior/key personnel during Yr03

Name: Alan D. Friedman
Changes: new
T2018-002, V Foundation
611711, The Andrew McDonough B+ Foundation

Name: Kenneth J. Pienta
Changes: completed
P30 CA006973 (role ended), National Institutes of Health
R01 CA211695 (role ended), National Institutes of Health
90079129, Prostate Cancer Foundation and Movember Foundation
new
18CHAL15, Prostate Cancer Foundation

Other organizations involved as partners

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

Not applicable.

9. APPENDICES

Suresh et al. (abstract of presentation at the Annual Meeting of the Society for Immunotherapy of Cancer, Nov. 8, 2019).

[Abstract for Poster presented on November 8, 2019 at the Annual Meeting of the Society for Immunotherapy of Cancer held in Washington, D.C.]

Rahul Suresh, David J. Barakat, Theresa Barberi, Lei Zheng, Elizabeth M. Jaffee, Kenneth J. Pienta, and Alan D. Friedman
Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

NF-kB p50-deficient immature myeloid cell (p50-IMC) adoptive transfer slows the growth of murine prostate and pancreatic ductal carcinoma

Background: NF-κB p50 binds DNA, but unlike p65, lacks a trans-activation domain and recruits co-repressors. Macrophages and dendritic cells lacking NF-κB p50 are skewed towards a pro-inflammatory phenotype, with increased cytokine expression and enhanced T cell activation; additionally, murine melanoma, fibrosarcoma, colon carcinoma, and glioblastoma grow slower in $p50^{-/-}$ mice. Given these data, we evaluated efficacy of p50-deficient immature myeloid cells (p50-IMC) adoptively transferred into tumor-bearing hosts. Immature cells were utilized to maximize tumor localization, and pretreatment with 5-fluorouracil (5FU) was examined due to its potential to impair marrow production of myeloid cells, to target tumor myeloid cells, and to potentially release tumor neoantigens.

Methods: WT-IMC or p50-IMC were generated by culturing lineage-negative marrow cells from WT or $p50^{-/-}$ mice in media containing TPO, SCF, and FL for six days followed by M-CSF for one day on ultra-low attachment plates. Mice inoculated with Hi-Myc prostate cancer (PCa) or K-Ras^{G12D} pancreatic ductal carcinoma (PDC)-luciferase cells received 5FU followed five days later by three doses of 1E7 IMC every three to four days. Some groups also received four doses of anti-PD-1 antibody twice weekly alone or with p50-IMC.

Results: PCa grew slower in $p50^{-/-}$ mice, and absence of host p50 led to prolonged survival of mice inoculated orthotopically with PDC. 5FU followed by p50-IMC slowed PCa and PDC tumor growth ~3-fold in contrast to 5FU followed by WT-IMC, 5FU alone, or p50-IMC alone. Slowed tumor growth was evident for 93% of PCa tumors but only 53% of PDC tumors. In PCa, p50-IMC predominantly generated tumor and draining lymph node $F4/80^{+}$ macrophages, but also $CD11b^{+}F4/80^{-}CD11c^{+}$ conventional dendritic cells. A subset of tumor and nodal macrophages co-expressed Ly6C and MHCII and had reduced MR compared to host macrophages, collectively indicating a pro-inflammatory phenotype. p50-IMC also produced a 5-fold increase in activated PCa tumor CD8 T cells, and antibody-mediated CD8 T cell depletion obviated slower tumor growth induced by 5FU followed by p50-IMC. Anti-PD-1 markedly slowed PCa growth but had little efficacy against PDC, whereas anti-PD-1 combined with p50-IMC slowed PDC tumor growth to prolong survival more effectively than either alone in an initial experiment.

Conclusions: 5FU followed by p50-IMC slows the growth of murine prostate and pancreatic ductal carcinoma and depends upon CD8 T cell activation. Deletion of p50 in patient-derived marrow $CD34^{+}$ cells and subsequent production of IMC for adoptive transfer may contribute to the therapy of these and additional cancers, alone or with additional immunotherapies.