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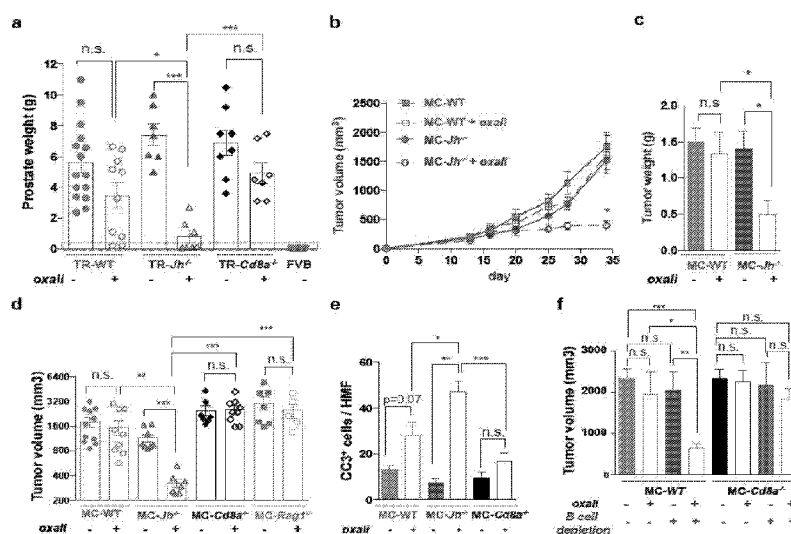


FIGURE 1

(57) **Abstract:** The invention provides a method for treating cancer in a subject in need thereof, wherein said subject comprises cancer tissue that 'contains epithelial cancer cells and immunosuppressive 8 cells, and wherein said method comprises administering to said subject a therapeutically effective amount of a) one or more first composition that pauses- immunogenic cell death and/or of said epithelial cancer cells, and b) one or more second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer.

NOVEL CHEMOIMMUNOTHERAPY FOR EPITHELIAL CANCER

This application claims priority to co-pending U.S. provisional Application Serial No. 62/134,258, filed on March 17, 2015, which is herein incorporated by reference.

GOVERNMENT INTEREST

This invention was made with government support under grant No. R01 CA127923 awarded by National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The invention relates to methods for treating cancer in a subject in need thereof, wherein said subject comprises cancer tissue that contains epithelial cancer cells and immunosuppressive B cells, and wherein said method comprises administering to said subject a therapeutically effective amount of a) one or more first composition that causes immunogenic cell death and/or of said epithelial cancer cells, and b) one or more second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer.

BACKGROUND OF THE INVENTION

Malignant progression and the effectiveness of cancer therapy are strongly modulated by innate and adaptive immunity^{4,33}. Whereas myeloid cells and their inflammatory cytokines promote tumorigenesis and confer therapy resistance^{21,33}, B and T lymphocytes exert both pro- and anti-tumorigenic effects^{7,34,35}. Three different strategies can unleash the powerful anti-cancer activity of tumor-infiltrating T (TIL) cells: immune checkpoint inhibitors^{36,37}, adoptive T cell therapy³⁸, and, in part, immunogenic chemotherapy^{1,4}.

Cancer is characterized by multiple genetic alterations and loss of normal cellular regulatory processes. This results in expression of tumor antigens, which can be presented by MHC molecules to activate T cells^{1,2}. However, the microenvironment of advanced tumors establishes T cell tolerance, which contributes to malignant progression. Despite significant progress in understanding how T cell tolerance is implemented and maintained^{1,3}, the critical rate-limiting mechanism operating in each cancer type remains unknown. Cancer-specific T cells must infiltrate and undergo activation within the tumor microenvironment, otherwise they cannot eradicate the malignancy. Indeed, new therapeutics

that overcome tolerogenic mechanisms are effective in certain human cancers¹. Immune mechanisms also affect the outcome of chemotherapy⁴. Importantly, certain chemotherapeutic agents (oxaliplatin, anthracyclines) stimulate cancer-specific immune responses by either inducing immunogenic cell death (ICD) or immune effector mechanisms^{5,6}. For example, in castrate-resistant prostate cancer (CRPC), B lymphocytes that are recruited by CXCL13 into prostate tumors promote development of CRPC that resists androgen ablation therapy, in a manner dependent on activation of an IKK α -Bmi1 module, needed for survival and proliferation of prostate cancer (PC) progenitor cells, by lymphotoxin (LT) expressing B cells^{7,8}.

Thus, what is needed are methods for treating tumors and reducing tolerance to cancer chemotherapy, which otherwise contributes to malignant progression.

SUMMARY OF THE INVENTION

The invention provides a method for treating epithelial cancer in a mammalian subject in need thereof, wherein said subject comprises cancer tissue that contains epithelial cancer cells and immunosuppressive B cells, and wherein said method comprises administering to said subject a therapeutically effective amount of (a) one or more first composition that causes immunogenic cell death, and (b) one or more second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer. In one embodiment, said first composition that causes immunogenic cell death is selected from the group consisting of oxaliplatin, cyclophosphamide, and mitoxantrone, anthracyclines, and bortezomib. In another embodiment, said first composition that causes immunogenic cell death comprises oxaliplatin. In a further embodiment, said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer comprises an antibody that specifically binds to said immunosuppressive B cells. In yet another embodiment, said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer comprises anti-CD19 antibody and anti-CD20 antibody. In a further embodiment, said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer comprises anti-PDL1 antibody. In another embodiment, said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer comprises anti-CD19-CAR T cells. In another embodiment, said immunosuppressive B cells comprise TGF beta receptors, and wherein said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer

is a compound that inhibits binding of said TGF beta receptor to a TGF beta receptor ligand. In a further embodiment, said second composition that inhibits binding of said TGF beta receptor to a TGF beta receptor comprises ALK5 inhibitor. In another embodiment, said epithelial cancer is selected from the group consisting of prostate cancer, liver cancer, bladder cancer, lung cancer, and cutaneous melanoma. In another embodiment, said prostate cancer is selected from the group consisting of castrate-resistant prostate cancer (CRPC), recurrent prostate cancer, and metastatic prostate cancer. In a further embodiment, said epithelial cancer is selected from the group consisting of primary cancer, recurrent cancer, and metastatic cancer. In another embodiment, said administering comprises administering said first composition and said second composition substantially simultaneously. In another embodiment, said administering comprises administering said first composition and said second composition sequentially in any order. In another embodiment, said administering comprises administering said first composition and said second composition substantially simultaneously and sequentially in any order.

The invention also provides a method for reducing prostate cancer in a subject in need thereof comprising,

- a) administering to said subject a therapeutically effective amount of an immunogenic chemotherapeutic, and
- b) treating said subject with one or more compound that results in one or more of
 - i) reducing the number of IgA⁺ B cells and/or immunosuppressive + B cells in said prostate cancer and/or in other epithelial cancer that contain immunosuppressive B cells,
 - ii) inhibiting the pathways through which the immunosuppressive B cells suppress T cells like PD-L1 and IL-10,
 - iii) reducing serum IgA, and
 - iv) reducing binding of TGF beta receptor to one or more TGF beta ligand, wherein said TGF beta receptor is comprised in B cells in said prostate cancer and/or other epithelial cancer.

In one embodiment, said immunogenic chemotherapeutic comprises oxaliplatin. In one embodiment, said prostate cancer is therapy-resistant prostate cancer. In one embodiment, said steps of administering said immunogenic chemotherapeutic and of treating said subject are concomitant. In one

embodiment, said steps of administering said immunogenic chemotherapeutic and of treating said subject are not concomitant.

The invention also provides a method of treating prostate cancer in a subject comprising,

- a) reducing the number of B cells in said prostate cancer; and
- b) administering to said subject a chemotherapeutic.

The invention further provides a method of treating prostate cancer in a subject comprising,

- a) treating B cells in said prostate cancer with an antibody; and
- b) administering to said subject a chemotherapeutic.

The invention additionally provides a method of treating prostate cancer in a subject comprising,

- a) administering a chemotherapeutic to said subject;
- b) treating B cells in said prostate cancer with an antibody. In one embodiment, said antibody binds to IgA⁺ B cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: B cells attenuate the anti-tumorigenic activity of low dose oxaliplatin. **a**, *TRAMP* (FVB) mice (*TR*-WT, *TR-Jh*^{-/-}, and *TR-Cd8a*^{-/-}; n=7-15 per group) received weekly oxaliplatin (6 mg/kg), starting at week 16 (see Fig. 11a; late treatment). After 4 weeks, mice were sacrificed and prostate weights measured. Dashed red line = prostate weight of naïve controls. **b**, Tumor growth in mice s.c. transplanted with MC cells and treated with oxaliplatin as in Fig. 11f (late treatment) or 5% dextrose (n=7-11 per group). **c**, Weights of MC tumors after treatment with oxaliplatin or vehicle (n=5-7 per group). **d**, Mice of indicated genotypes bearing s.c. MC tumors (n=7-11 per group) were treated as above. After 3 cycles, tumor volumes (mm³) were determined. **e**, MC tumors from above mice were stained for CD45 (green) and cleaved caspase 3 (CC3; red) (n=4-6 per group). **f**, Numbers of CC3⁺ CD45⁺ cells per high magnification field (HMF; 200×) in tumors from panel (e). Magnification bars: 100 μm. **g**, MC tumors were raised in WT (**left**), or *Cd8a*^{-/-} (**right**) mice. Sixteen days after inoculation, B cells were depleted with antibodies against CD19, CD20, CD22 and B220. Four days after first twice-weekly antibody treatment, mice received weekly oxaliplatin (n=4-7 per group, total: 42), and sacrificed 3 weeks later. Tumor volumes were analyzed by Kruskal-Wallis test: P=0.007**. Results are means ± s.e.m. Mann-Whitney and t tests were used to determine significance indicated as *P, 0.05; **P, 0.01; ***P, 0.001.

Figure 2: B cells inhibit oxaliplatin-induced T cell activation. **a**, CD8⁺ cell counts in TRAMP prostates (WT, *Jh*^{-/-}; n=4-6 per group) collected from mice treated as in **1a**, analyzed by flow cytometry and normalized to CD45⁺ cells. **b**, Mice (n=6-8 per group) bearing MC tumors were analyzed as above for CD8⁺ cells in spleens and tumors after 3 chemotherapy cycles. **c**, MC tumors from (b) were stained for CD8 (Fig. 12b), and tumor-infiltrating CD8⁺ cells were counted in 3-4 HMF (200×) per tumor (n=4-5 tumors per group). **d, e**, Q-RT-PCR analysis of *Perforin* and *Ifnγ* mRNA in MC tumors collected as in (b) (n=4-7). **f**, Flow cytometry for TNF and IFNγ in CD8⁺ cells from MC tumors of mice treated as above (n=6-8) and re-stimulated *in vitro* with tumor cell lysate. **g-i**, Flow cytometry of GrzB and Ki-67 (**g**), PD-1 and Tim-3 (**h**) and BTLA (**i**) in CD8⁺ T effector cells (CD8⁺CD44⁺; **g,h**) or total CD8⁺ cells (**i**) from spleens and tumors of MC inoculated mice. Results are either percentages of positive cells in tumoral CD8⁺ cells or mean fluorescence intensities (MFI) and are means ± s.e.m of three independent experiments (n=6-8 mice per group). Mann-Whitney and t tests were used to determine significance shown as above.

Figure 3: Oxaliplatin induces infiltration of prostate tumors with IgA⁺PD-L1⁺IL-10-producing plasmacytes. **a**, B220⁺CD19⁺ B lymphocytes in 20 weeks old TRAMP prostates after 4 oxaliplatin cycles (n=5-7 per group) normalized to prostate weights. **b,c**, Flow cytometry of B220, CD19, CD138 and IgA in tumoral B cells from (a). Values are % of tumoral CD45⁺ (**b**) or CD19⁺ (**c**) cells. **d**, MC tumors (n=4-5 per group) stained for αSMA (green) and IgA (red). Arrows: IgA⁺ cells whose number per HMF is shown on the right. **e**, Flow cytometry of p-SMAD2/3 in tumor-infiltrating B cells (n=3-4 per group). **f**, *Il10* mRNA in MC tumors (n=5-6 per group). **g**, Tumor-infiltrating IL-10⁺CD19⁺B cells in MC-WT mice, shown as percentages of CD45⁺ cells. **h,i**, Percentages of IL-10-producing cells in tumoral CD19⁺IgA⁺ (**h**) and CD19⁺IgA⁻ (**i**) cells from MC-WT mice. **j**, IL-10 expression by IgA⁺ and IgA⁻ B cells in MC-tumors (n=4-6 per group). **k**, Flow cytometry of PD-L1 and FAS-L in B cells from TRAMP tumors. **l**, *Pdli* mRNA in MC tumors (n=5-6 per group). **m**, Low (n=5) and high (n=5) risk human PC specimens stained with IgA (red) and αSMA (green) antibodies. Arrows: IgA⁺ cells. All results are means ± s.e.m of at least three independent experiments. Magnification bars: 100 μm. Mann-Whitney and t tests were used to calculate statistical significance shown as above.

Figure 4: TGFβR signaling and IgA class-switch recombination are required for development of immunosuppressive plasmacytes. **a**, MC tumors were raised in WT, *Tgfb2*^{ΔB} or *Iga*^{-/-} mice until 400 mm³ in size, after which mice were given 3 weekly oxaliplatin cycles (n=5-11 per

group, total: 48). Tumor volumes at treatment end were analyzed (Kruskal-Wallis test: $P=0.0004^{***}$). **b, c**, Tumor-infiltrating $CD19^+$ (**b**) and IgA^+ (**c**) cells, shown as percentages of tumoral $CD45^+$ cells (**b**) or total vital cells (**c**) ($n=4-7$ per group). **d**, Serum IgA in treated and untreated MC-WT and MC-*Tgfb β 2^{ΔB}* mice ($n=5-8$ per group). Naïve tumor-free WT and *Tgfb β 2^{ΔB}* mice are shown as controls. **e**,
 5 Frequency of tumor-infiltrating $CD8^+$ T cells in mice from (**a**). **f**, $CD8^+$ T cells (5×10^6 per well) from MC tumors in (**a**) were re-stimulated for 4 hrs with either MC lysate (left) or PMA/ionomycin (right) and analyzed for the indicated markers. Percentages of $IFN\gamma^+CD107a$ -expressing $CD8^+$ T cells within tumoral $CD8^+$ cells are shown ($n=4-7$ mice per group). **g**, B cells (5×10^6 ; 98% pure) from WT, *Pd1/2^{-/-}* and *Il10^{-/-}* mice were transferred into MC tumor-bearing *Jh^{-/-}* mice (16 days after inoculation)
 10 that received oxaliplatin 2 days later. Tumor volumes were determined on day 30 ($n=4-6$ mice per group). **h**, Tumor infiltrating $CD8^+$ T cells from *Jh^{-/-}* MC tumor-bearing mice transplanted with B cells and treated as above were re-stimulated for 4 hrs with PMA/ionomycin before analysis of $IFN\gamma$ and $CD107a$ ($n=4-6$ mice per group). Results are means $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance between two groups.

15 **Figure 5: Adoptively transferred B cells inhibit T cell-dependent tumor eradication. a**, Sixteen weeks old *TRAMP;Rag1^{-/-}* mice received weekly oxaliplatin. One day after 1st treatment, CFSE-labeled splenocytes from WT (B and T cells, SP-WT) or *Jh^{-/-}* (T but no B cells, SP-*Jh^{-/-}*) mice were adoptively transferred (ACT) into tumor-bearing mice (4-5 per group; 5×10^6 T cells per mouse). **b, c**, After 3 more oxaliplatin cycles, mice were sacrificed, their prostates photographed (**b**) and tumor
 20 weight measured (**c**). **d**, Frequency of adoptively transferred $CD19^+$ cells amongst $CD45^+$ cells in spleens and prostates 30 days after ACT. **e,f**, Serum IgA and IgG in both ACT groups and FVB-WT mice. **g,h** Serum anti-SV40-Tag IgA and IgG concentrations in indicated mouse strains with or without ACT and/or oxaliplatin treatment. **i**, Frequency of $CD8^+$ cells amongst $CD45^+$ cells in *TRAMP;Rag1^{-/-}* prostates after ACT and oxaliplatin treatment. **j**, MC tumor-bearing *Rag1^{-/-}* mice were treated with
 25 oxaliplatin. One day later, mice (4-5 per group) received 5×10^6 T cells from WT mice immunized with MC cell extract without or with 5×10^6 B cells (98% pure) from WT or *Tgfb β 2^{ΔB}* mice. After 2 more oxaliplatin cycles, mice were sacrificed and tumor volumes determined. **k**, Flow cytometry of tumoral $CD8^+$ cells in above mice. Cells were re-stimulated for 4 hrs with PMA/ionomycin before analysis. Shown are percentages of $IFN\gamma$ -expressing cells in total $CD8^+$ cells ($n=5-8$ per group, small
 30 tumors were pooled). **l**, Serum IgA in above mice. Results are means $\pm$ s.e.m. Mann-Whitney and t tests were used to determine significance. n.d. not detectable.

Figure 6. Treatment schemes and characterization of tumors and mouse survival before and after treatment. **a**, Early and late treatment schemes for *TRAMP* mice. **b**, *TRAMP* mice (n= 3-6 for each group) were subjected to early oxaliplatin treatment as described in (**a**) and prostate weights were determined at 14 weeks, one week after completion of 4 treatment cycles. Dashed red line indicates prostate weight of tumor-free controls (n=33 in total). **c-e**, Histopathology of *TRAMP* tumors. **c**, Representative images of H&E stained prostate sections from *TRAMP* mice are shown. Magnification bars: 100 μ m. PIN, prostatic intraepithelial neoplasia; WD, well differentiated adenocarcinoma; PD, poorly differentiated adenocarcinoma. **d**, Histopathological assessment of early *TRAMP* tumors in WT and *Jh^{-/-}* mice without or with oxaliplatin treatment. The percentages of the different histotypes shown in (**c**) are depicted (n=3-7 per group). **e**, Histopathological assessment of late *TRAMP* tumors from WT and *Jh^{-/-}* mice without or with oxaliplatin treatment. The percentages of the different histotypes are depicted (n=3-7 per group). Fisher chi-square analysis was used to calculate statistical significance in (**d**) and (**e**). **f**, Early and late treatment schemes for mice bearing s.c. MC tumors. **g**, MC cells were s.c. transplanted into WT and *Jh^{-/-}* mice (n= 3-7 per group) that were subjected to early oxaliplatin treatment when tumor volume was 100 mm³. 48 hrs after completion of 3 treatment cycles, mice were sacrificed and tumor volumes (mm³) were measured (n=19 in total). Prostate weight in (**g**) is shown in a Log 2 scale. **h,i**, MC tumors (n=3-5 per group) grown in WT, *Jh^{-/-}* and *Cd8a^{-/-}* mice were stained for CD45 (green) and p- γ H2AX (red), and the p- γ H2AX⁺ foci in CD45⁺ cells were enumerated (**i**). Magnification bars: 100 μ m. All results are means $\pm$ s.e.m. **j**, Representative images of s.c. MC tumors (n=5-6 per group) from WT and *Jh^{-/-}* mice, with or without oxaliplatin treatment stained for α SMA (green) and CD31 (red). **k, l**, Frequency of α SMA (**k**) and CD31 (**l**) positive cells within tumors from (**j**). Shown are median values $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance indicated by *P , 0.05; **P , 0.01; ***P , 0.001. **m**, *TRAMP* mice (WT, *Cd8a^{-/-}* or *Jh^{-/-}*; n=6-14 per group) were treated weekly with low-dose oxaliplatin. Moribund mice were sacrificed, and survival was compared by Kaplan-Meyer analysis and significance was determined (WT: n.s.; *Cd8a^{-/-}*: n.s.; *Jh^{-/-}*; p<0.002; **). **n**, No statistically significant differences in survival were found between WT and *Jh^{-/-}* or *Jh^{-/-}* and *Cd8a^{-/-}* mice without treatment. **o**, Significant differences in survival times were observed between all three oxaliplatin-treated groups (WT, *Cd8a^{-/-}* or *Jh^{-/-}*; indicated on the right). **p**, Survival curves for all of the groups before and after oxaliplatin treatment. Significant differences in survival times are indicated on the right.

Figure 7. B cells attenuate oxaliplatin-triggered CTL activation. **a**, Flow cytometry of CD8⁺ T lymphocytes in prostates of 20 weeks old *TRAMP* mice after 4 cycles of oxaliplatin treatment (n=5-7 per group) normalized to prostate weights. **b**, Late s.c. MC tumors from WT and *Jh*^{-/-} mice were stained for CD8 and analyzed by immunofluorescent microscopy. In the upper left areas (white square), single CD8 staining (green) without DAPI counterstain is shown. Quantitation of CD8⁺ cells is shown in **Fig. 2c**. Magnification bars: 100 μ m. **c,d**, Late s.c. MC tumors were analyzed by flow cytometry for CD4⁺ lymphocytes in spleens (**c**) and tumors (**d**) after 3 oxaliplatin treatment cycles (n=4-7 per group). The results show percentages of CD4⁺ cells in the CD45⁺ population. **e**, Flow cytometric analysis of IFN γ expression by CD8⁺ cells in MC tumors from WT and *Jh*^{-/-} mice treated as above (n=6-8) and re-stimulated *in vitro* with tumor cell lysate. Representative panels are shown in **Fig. 2f**. The results show percentages of IFN γ -producing CD8⁺ cells. **f**, Flow cytometry of STAT1 phosphorylation in CD8⁺ cells from MC tumors of treated and untreated WT, and *Jh*^{-/-} mice (for isotype controls, see **E.D. Fig. 10s**). The results are summarized in the right panel (n=3 mice per group). **g,h**, Flow cytometry of PD-1 and Tim-3 expression by CD8⁺ effector cells (CD8⁺CD44⁺ cells) in MC tumors (**g**) and spleen (**h**) from WT or *Jh*^{-/-} mice with or without oxaliplatin treatment. Shown are percentages of the corresponding CD8⁺ T cells in the CD8⁺CD44⁺ population (n=3-5 per group). **i**, The experimental scheme for B cell immunodepletion in tumor-bearing mice. MC tumors were raised in WT or *Cd8a*^{-/-} mice, 16 days after s.c. tumor cell inoculation. B cells were depleted by twice weekly administration of antibodies directed against CD19, CD20, CD22 and B220. Four days after first antibody treatment, mice were treated with oxaliplatin (n=4-7 mice per group, total: 44). After 3 weekly chemotherapy cycles, mice were sacrificed. **j,k**, Flow cytometry analysis of tumor-infiltrating CD45⁺CD8⁺ T cells stained for IFN γ (**j**) or IFN γ and TNF (**k**) after *in vitro* restimulation with PMA/ionomycin (n=4-6 mice per group). **l-n**, Flow cytometry analysis of CD19⁺ (**l,m**) and IgA⁺ (**n**) cells in spleens and tumors isolated from the WT mice described above, confirming depletion of CD19⁺ B cells and oxaliplatin-induced IgA⁺ cells in spleen and tumors. **o**, Serum IgA concentrations in the mice described in **i** (n=3-5 per group). **p**, Flow cytometry analyses of CD19⁺ B cells in tumors isolated from *Cd8a*^{-/-} mice subjected to B cell depletion or not, confirmed the efficient depletion of tumoral CD19⁺ B cells. All results are means $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance indicated by *P, 0.05; **P, 0.01; ***P, 0.001.

Figure 8: Immunogenic chemotherapy induces tumor infiltration by immunosuppressive CD19⁺CD20^{low}B220^{low}IgA⁺ B cells. **a,b**, MC tumors (n=4-9 per group) raised in WT mice without or

with oxaliplatin treatment were stained for B220 (a), and tumor-infiltrating B220⁺ cells per HMF were enumerated (b). In panel a, single B220 staining (above) and combined staining B220/DAPI (below) are shown. Magnification bars: 100 μ m. c,d, The flow cytometry plots and gating strategy for analysis of splenic B cell populations using CD19, IgA, B220, CD138 and CD20 antibodies. Results from WT mice bearing MC tumors are shown in panel (c) and from oxaliplatin-treated mice in panel (d) (n=8 mice per group). Oxaliplatin treatment modestly increased the amount of splenic IgA⁺ cells. Splenic IgA⁺ cells expressed CD138 as expected and showed lower levels of B220 and CD20, in either control or oxaliplatin-treated mice. e, The gating strategies for analysis of tumoral B cells using CD19, IgA, B220 and CD138 antibodies. Results from MC tumors in two representative oxaliplatin-treated WT mice are shown (n=8 mice per group), demonstrating the presence of IgA⁺ cells in oxaliplatin-treated tumors with a typical CD138⁺B220^{low} phenotype. f-i, Flow cytometry plots and gating strategies for analysis of tumoral B cell populations using CD19, B220, CD138, IgA and PD-L1 antibodies. Results from WT mice bearing MC tumors without (f) or with oxaliplatin treatment (g) (n=6 mice per group) and IgA^{-/-} mice bearing MC tumors without (h) or with oxaliplatin treatment (i) (n=6 mice per group) are shown. Oxaliplatin treatment increased the amount of tumoral IgA⁺CD138⁺B220^{low}PD-L1⁺ cells in WT mice. j, Flow cytometric analysis of PD-L1 and IL-10 expression in IgA⁺B220⁻ and B220⁺IgA⁻ B cells from oxaliplatin-treated TRAMP tumors (n=4). k, Flow cytometric analysis of IgA and CD138 expression by TRAMP tumor-infiltrating B cells. Shown are percentages of IgA⁺ cells amongst all tumor-infiltrating CD19⁺CD138⁺ cells. l, WT mice bearing MC tumors were treated with oxaliplatin as above. Two days after the first or last oxaliplatin cycle, mice were sacrificed, tumors were isolated and analyzed by flow cytometry as indicated (n=6 per group). After dead cell exclusion, tumor-infiltrating B cells were stained with CD19, CD20, B220, IgA and IgM antibodies. Shown are the results for control (left panels), one cycle (middle panels), and 3 cycles (right panels) of oxaliplatin treatment, demonstrating the presence of tumoral IgA⁺ cells with a CD19⁺CD20^{low}B220^{low} IgA⁺ cell phenotype within 48 hrs after oxaliplatin treatment.

Figure 9: Immunogenic chemotherapy induces tumoral and systemic IgA production through class switch recombination. a, *Ex vivo* analysis of IgA released by tumor single cell suspension isolated from oxaliplatin-treated TRAMP tumors. Single cell suspension from non-treated tumors and culture medium without cells were used as controls. b,c, Serum IgA (b) and IgG (c) in treated and untreated *TRAMP* mice and age-matched naïve FVB controls (n=7-14 per group). d, Serum IgA amounts in control or oxaliplatin-treated mice bearing MC tumors (n=5-7 per group) were

determined and compared to age-matched naïve FVB controls (n=7). **e**, Serum IgG amounts in control or oxaliplatin-treated mice bearing MC tumors (n=5-7 mice per group) were determined and compared to age-matched naïve FVB controls. **a-e**, Results are means $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance. **f,g**, Immunofluorescence analysis of activation-induced cytidine deaminase (AID, green) and IgA (red) expression in spleen (**f**, used as a positive control) and MC tumors from oxaliplatin-treated WT mice (**g**). Magnification bars: 10 μ m except in bottom left panel where it is 100 μ m. Arrows point to IgA⁺AID⁺ cells. Shown are representative results of spleens and tumors isolated from 4 mice per group. **h**, Q-RT-PCR analysis of *Tgfb1* mRNA in MC tumors raised in WT or *Jh*^{-/-} mice without or with oxaliplatin treatment (n=3-7 mice per group). Results are means $\pm$ s.e.m. **i**, Flow cytometry of SMAD2/3 phosphorylation in MC tumor-infiltrating B cells from WT mice before and after oxaliplatin treatment (n=4 per group). Shown are the mean fluorescence intensities (MFI) and percentages (see **Fig. 3e**). **j**, Flow cytometry of SMAD2/3 phosphorylation and PD-L1 in MC tumor-infiltrating B cells from WT mice before and after oxaliplatin treatment (n=4 per group). Shown are the percentages of PD-L1⁺p-SMAD2/3⁺ cells within CD45⁺CD19⁺ cells. **k**, Q-RT-PCR analysis of *Il21* mRNA in MC tumors raised in WT or *Jh*^{-/-} mice without or with oxaliplatin treatment (n=4-7 mice per group). Chemotherapy-induced *Il21* mRNA mainly in WT mice. **l,m**, Flow cytometry of tumor-infiltrating B cells stained for phospho-STAT3 and IL-10 (n=5-6 per group) before and after oxaliplatin treatment. **n**, Flow cytometry analysis of β -actin mRNA, IL-10 protein and *Il10* mRNA in MC tumor-infiltrating IgA⁺ cells using PrimeFlow™ RNA technology (pooled data of 4 mice per group, after oxaliplatin treatment). Left panel: β -actin mRNA gated on CD45⁺ cells; middle panel: *Il10* mRNA and IL-10 protein expression after 1 hr stimulation with PMA/ion/LPS gated on IgA⁺ cells, right panel: *Il10* mRNA and IL-10 protein expression after 5 hrs stimulation with PMA/ion/LPS, gated on IgA⁺ cells. **o,p**, Flow cytometric analysis of tumor-infiltrating B cells in *TRAMP* mice (n=4-5 per group) stained for CD19, B220, IL-10 and LT α β (**o**). The percentage of tumor-infiltrating LT α β ⁺ cells amongst all tumor-infiltrating B cells was determined (**p**). **q**, Flow cytometric analyses of CD5 expression by B cells from spleen and MC-tumor of WT mice after oxaliplatin treatment (n=4-5 per group). Shown are means $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance indicated by *P, 0.05; **P, 0.01; ***P, 0.001.

Figure 10: Immunogenic chemotherapy or B cell deficiency has marginal effects on Tregs, NK and myeloid cells. **a,b**, Q-RT-PCR analyses of *Nos2* (**a**) and *Arg1*(**b**) mRNA content of MC tumors (n=4-7 mice per group). Chemotherapy induced *Nos2* and *Arg1* expression in WT and *Jh*^{-/-}

mice and no significant and consistent differences were found between both groups. **c,d**, Q-RT-PCR analyses of *Il12p40* (**c**), *Il12p35* (**d**) mRNA in MC tumors grown in WT and *Jh*^{-/-} mice (n=4-6 mice per group). **e-i**, Flow cytometry analyses of tumor-infiltrating or splenic lymphocytes and monocytes: tumoral Nk1.1⁺ cells (**e**), tumoral CD11b⁺CD11c⁺MHCII⁺ cells (**f**), tumoral CD11b⁺GR-1⁺ cells (**g**), CD4⁺FoxP3⁺ cells (**splenic, h; tumoral, i**). Cells in panels **e-i** are from tumor-bearing mice subjected to oxaliplatin treatment and/or B cell depletion as indicated (B cell depletion + oxaliplatin; n=4-6 mice per group). Results are means ± s.e.m. Mann-Whitney and t tests were used to calculate statistical significance.

Figure 11: Analyses of B and T cells in human prostate cancer specimens. a-h, Tissue microarrays of tumor and non-tumor tissue from 110 PC patients were stained for CD8 and CD20 (5-6 spots per patient = 3-4 tumor tissue + 2 non-tumor tissue). **a,b**, Representative examples of CD8 (**a**) and CD20 (**b**) IHC of PC tissue microarrays (left). Magnification bar: 200 μm. Right, computer assisted image analysis with *ad hoc* developed image software. Tumor tissue is represented in yellow and CD8⁺ and CD20⁺ cells are represented in red. The percentages of immune reactive area (IRA) occupied by CD8⁺ or CD20⁺ cells are shown. Magnification bars: 200 μm. **c,d**, comparison of CD8 and CD20 IRAs in matched non-tumor and tumor tissues from each early stage PC (E-PC) patient (n=87). **e-h**, Patients were divided into three subgroups: E-PC (n=86); therapy-resistant-PC (TR-PC; n=9), and metastatic-PC (M-PC; n=15). **e**, CD8⁺ cell infiltration into tumor tissues of the different groups. **f**, CD20⁺ cell infiltration into tumor tissues of the different groups. **g**, The CD8⁺/CD20⁺ ratio for the different groups. **h**, The CD20⁺/CD8⁺ ratio for the different groups. Each dot represents one patient. Line indicates the median value. Mann-Whitney test was used to calculate statistical significance between the two groups. Kruskal-Wallis test was used to calculate statistical significance between the three groups. **i**, IHC analysis of low risk (n=5) and high risk (n=5) human PC specimens using IgA (red) and αSMA (black). Nuclei were counterstained with hematoxylin. Magnification bar: 100 μm. **j**, IF analysis of human PC showing IL-10 (red) –expressing IgA⁺ (green) CD138⁺ (turquoise) plasma cells (n=6). Representative images are shown. White arrow indicates IL-10-expressing IgA⁺ cells. Magnification bars: 50 μm. **k**, Human normal prostate (n=3-5), and human PC (n=5), were stained for IgA and CD8. Typical images are shown. Red and green arrows indicate IgA⁺ and CD8⁺ cells, respectively. Magnification bar: 100 μm. **l**, Human normal prostate (n=3), and human PC (n=5), were stained for IgA (red arrow) and CD20 (green arrow). Magnification bar: 100 μm. **m**, Flow cytometric analysis of human prostate tumor-infiltrating CD19⁺ B cells and IgA⁺ cells. The

percentages of IL-10-expressing B cells in CD19⁺IgA⁺ (2 different samples) and CD19⁺IgA⁻ B cells are shown. **n**, Summary of results obtained from human blood samples taken from healthy donors (n=3) and PC patients (n=5) and prostate tissue specimens (benign, malignant; n=4) analyzed by flow cytometry for IL-10 expression in CD19⁺IgA⁺ and CD19⁺IgA⁻ B cells. **o,p**, Tissue microarrays from 110 PC patients (described above) were stained for IgA and CD138. Patients were divided into three subgroups: E-PC (n=86); TR-PC (n=9), and M-PC (n=15). **(o)** Representative images of IgA (immunoperoxidase) and CD138 (alkaline phosphatase) double staining of tumor tissues from each group. CD138⁺ and IgA⁺ double positive cells in the PC stroma are indicated by the white arrows (hematoxylin counterstain). Magnification bar: 100 μ m. **p**, Frequencies of IgA⁺ and CD138⁺ double positive cells in the tumor stroma of the different PC patient groups. **q**, PC patient specimens were divided into two groups: IgA^{low} (n=64) and IgA^{hi} (n=46). Shown is the CD8⁺/CD20⁺ ratio for each group. Each dot represents one patient. Line indicates the median value. **r**, IgA mRNA expression (*IGHA1*) is significantly elevated in human PC tissue relative to healthy or benign prostate tissue in 5 out of 15 studies evaluated via Oncomine. Results from one significant study¹⁴ are presented. Chi square test and Fischer exact test were used to calculate statistical significance shown by *P, 0.05; **P, 0.01; ***P, 0.001.

Figure 12: Effects of TGF β R2, IgA, PD-L1 and IL-10 ablations on tumor-infiltrating lymphocytes. MC tumors were raised in WT, *Tgfb2^{AB}* or *Iga^{-/-}* mice (n= 5-11 per group). Mice were subjected to 3 cycles of late oxaliplatin treatment after which splenic (Spl) and tumoral (Tu) B cells were analyzed. After dead cell exclusion, splenic (**a,b**) and tumoral (**c,d**) B cells were stained with CD19, B220, IgA, IgG2a and IgG1 antibodies and analyzed by flow cytometry. **e**, Serum IgG concentrations in control or oxaliplatin-treated WT, *Tgfb2^{AB}* or *Iga^{-/-}* mice bearing MC tumors (n=5-9 per group). **f**, Flow cytometry of tumor-infiltrating CD19⁺ B cells from WT, *Tgfb2^{AB}* or *Iga^{-/-}* MC tumor-bearing mice (n=4-7 per group) analyzed for PD-L1 expression, revealing lower PD-L1 surface expression on *Tgfb2^{AB}* and *Iga^{-/-}* B cells after oxaliplatin treatment. **g**, Flow cytometry of tumor-infiltrating B220^{hi} B cells (left) and IgA⁺B220^{low} B cells (right) from WT, *Tgfb2^{AB}* or *Iga^{-/-}* MC tumor-bearing mice (n=4-7 per group) analyzed for IL-10 expression, revealing no difference in IL-10 expression by B220^{hi}IgA⁻ B cells in the corresponding groups, and lower IL10 expression by *Tgfb2^{AB}* B cells after oxaliplatin treatment compared to WT mice. Results are means $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance. **h**, The experimental scheme. WT mice bearing MC tumors were divided into four treatment groups (n=7-8 each group): 1) isotype control

(IgG2a); 2) oxaliplatin (weekly); 3) anti-PD-L1 (twice weekly); 4) oxaliplatin plus anti-PD-L1 (weekly and twice weekly, respectively). After 3 treatment cycles, mice were sacrificed and analyzed. **i**, Tumor growth curves of tumor-bearing mice and gross appearance of untreated and treated mice. Significance was determined by Mann-Whitney and t tests. **j**, Flow cytometric analysis for GrzB⁺ expression by tumor-infiltrating CD8⁺ T effector cells (CD8⁺CD44⁺) from MC tumor-bearing mice treated as described above. Results are shown either as percentages of GrzB⁺ cells amongst CD8⁺ T cells (black), or percentages of GrzB⁺CD8⁺CD44⁺ T cells amongst tumoral CD45⁺ cells (red). **k**, Flow cytometry of PD-L1 expression on tumor-infiltrating IgA⁺ CD19⁺ B cells in the different treatment groups. **l,m**, Serum IgA (**l**), and IgG (**m**) concentrations in the different treatment groups described in panel **h**. **n**, The experimental scheme for the experiment whose results are shown in **Fig. 4g,h**. B cells were isolated from WT, *Pd11/2*^{-/-} and *Il10*^{-/-} mice and 5×10^6 cells (purity 98%) were i.p. transferred into MC tumor-bearing *Jh*^{-/-} mice (16 days after MC cell inoculation). After 2 days (day 18), the mice were given 3 oxaliplatin treatment cycles and analyzed. **o**, Flow cytometric analysis of splenocytes after staining with CD45 and CD19 antibodies, confirming presence of B cells in the ABCT groups. Shown are percentages and absolute B cell numbers in spleen.

Figure 13: Low dose cisplatin treatment is devoid of immunogenic activity and low dose oxaliplatin does not affect gut barrier function. **a**, Flow cytometry of MC cells stained with Annexin V and propidium iodide 24 hrs after treatment with either oxaliplatin or cisplatin (both at 20 μ M). **b**, Flow cytometry analysis of MC cells treated as above and stained with antibody to the autophagy marker LC3A. **c-e**, MC tumors were raised in WT and *Jh*^{-/-} mice until 400 mm³ in size, after which the mice were treated with either cisplatin or oxaliplatin at 6 mg/kg (n=4-5 per group). After 3 weekly chemotherapy cycles, mice were sacrificed. **c**, Tumor weights; left panel: WT mice; right panel: *Jh*^{-/-} mice. **d, e**, Flow cytometry of tumor-infiltrating CD8 (**d**) and CD4 (**e**) cells. Left panel: WT mice, right panel: *Jh*^{-/-} mice. **f**, Gut permeability was measured in WT mice before and after low (LD) and high (HD) dose oxaliplatin treatment using orally administered fluorescein isothiocyanate (FITC)-dextran. Shown are FITC-dextran concentrations in serum (μ g/ml) (n=5 mice per group). **g**, Serum IgA concentrations in naïve WT (FVB) and *Tgfb2*^{ΔB} mice before and after oxaliplatin treatment. **h**, IgA staining of colon sections of untreated or LD oxaliplatin-treated WT mice. Magnification bars: 100 μ m. **i-k**, Flow cytometry of CD8⁺ (**i**), CD4⁺ (**j**) and Nk1.1⁺ (**k**) cells in spleens of naïve WT and *Tgfb2*^Δ mice without or with oxaliplatin treatment. All results are means $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance shown as *P, 0.05; **P, 0.01; ***P, 0.001.

Figure 14: Immunogenic chemotherapy supports adoptive T cell transfer only in the absence of B cells. **a**, The experimental scheme. Immunogenic TRAMP-C2 cells were s.c. inoculated into WT or *Terβ^{-/-}* mice. After 30 days, the mice were divided into 4 groups (n=4-5 each group): 1) control, 2) oxaliplatin (weekly), 3) ATCT, 4) ATCT plus oxaliplatin (weekly). The first oxaliplatin cycle was given at day 31. Two days after the second cycle, CD8⁺ T cells from *CD45.1xCD45.2* WT mice (3×10^6 cells) were transferred into tumor-bearing mice and this was followed by two more oxaliplatin cycles after which mice were sacrificed for analysis on day 59. **b**, Tumor volumes (mm³) **c,d**, Flow cytometric analysis of spleen (**c**) and tumor (**d**) cells after staining with CD45.1, CD45.2, CD8 and TCRαβ antibodies, confirming expansion of adoptively transferred T cells. **e**, tumor growth curves. **f**, The experimental scheme. Immunogenic TRAMP-C2 cells were s.c. inoculated into WT or *Rag1^{-/-}* x OT-1 mice (no B cells), that harbor CD8⁺ T cells specific for chicken ovalbumin which is not expressed by TRAMP-C2 cells. After 30 days, tumor-bearing *Rag1^{-/-}* x OT-1 mice were divided into 4 groups (n=3-4 mice per group): 1) control, 2) oxaliplatin treatment, 3) ATCT, 4) oxaliplatin treatment plus ATCT. The first oxaliplatin cycle was given at day 31. Two days after the second oxaliplatin cycle, CD8⁺ T cells (3×10^6) from *CD45.1xCD45.2* mice were adoptively transferred into tumor-bearing mice, which were sacrificed on day 59 and analyzed. **g**, Flow cytometric analysis of tumor-infiltrating cells stained with CD45.1, CD45.2, CD8 and TCRαβ antibodies, confirming infiltration of adoptively transferred T cells. **h**, Flow cytometric analysis of GrzB expression in adoptively transferred, tumor-infiltrating, CD8⁺ effector cells (CD45.1⁺CD8⁺CD44⁺) from tumor-bearing mice treated as above. **i**, Tumor volumes (mm³). **j**, tumor growth curves. **k-q**, Sixteen weeks old *TRAMP;Rag1^{-/-}* mice (no B and T cells) were treated with oxaliplatin (weekly). One day after the 1st treatment cycle, CFSE-labeled splenocytes from either WT (B and T cells, SP-WT) or *Jh^{-/-}* (T but no B cells, SP-*Jh^{-/-}*) mice were transferred into the tumor-bearing mice (5×10^6 T cells per mouse; 4-5 mice per group). **k, l**, After 6 days, one mouse from each group was sacrificed, and the proliferation of CD8⁺ (**k**) and CD4⁺ (**l**) T cells in bone marrow (BM), spleen and prostates was analyzed by CFSE staining and flow cytometry. **m-q**, After 3 more oxaliplatin cycles (4 weeks in total), the mice were sacrificed and analyzed. **m,n**, Flow cytometric analyses of CD19⁺ B lymphocytes for TIM-1 expression in spleens (**m**) and prostates (**n**) of above mice. **o-q**, Flow cytometric analyses of T lymphocytes. Percentages of CD8⁺ and CD4⁺ T cells in LN (**o**); spleens (**p**); prostates (**q**) of above *TRAMP;Rag1^{-/-}* mice. Red: splenocytes from WT mice (T and B cell transfer), blue: splenocytes from *Jh^{-/-}* mice (T cell transfer). **r**, The experimental scheme for **Fig. 5j**. MC tumor-bearing *Rag1^{-/-}* mice (no B and T cells)

were treated with oxaliplatin (weekly). One day after 1st oxaliplatin treatment, 5×10^6 T cells (negative selection) from WT mice immunized with MC cell lysate¹⁰ were adoptively transferred into tumor-bearing mice (4-5 mice per group), alone or in combination with 5×10^6 B cells from WT or *Tgfb β 2^{ΔB}* mice (purity 98%). After 2 more oxaliplatin cycles (3 weeks total), the mice were sacrificed and analyzed. **s**, Serum IgG analysis of above mice. **t**, Flow cytometric analysis of splenocytes after staining with CD45 and CD19 antibodies. All results are means $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance. Statistical significance is given by *P , 0.05; **P , 0.01; ***P , 0.001.

Figure 15: Analysis of lymphocytes and monocytes in tumor-free mice, gating strategies and staining controls. WT, *Jh^{-/-}*, *Iga^{-/-}* and *Tgfb β 2^{ΔB}* mice in the FVB background and WT, *Pd11/2^{-/-}*, *Il10^{-/-}* and *Iga^{-/-}* in the C57BL/6 background were analyzed for the distribution of immune markers. **a**, Spleen weights of WT, *Jh^{-/-}* and *Tgfb β 2^{ΔB}* mice in the FVB background. **b**, Flow cytometry of splenocytes for the following markers: CD3 (**left**), CD8 (**middle**), CD4 (**right**), gated on the splenic CD45⁺ population. **c**, Absolute cell numbers of splenic CD3⁺ (**left**), CD8⁺ (**middle**), and CD4⁺ (**right**) cells are shown (percentage $\times$ cell count of whole spleen). **d,e** Flow cytometry for TNF and IFN γ in CD8⁺ cells from tumor-free WT, *Jh^{-/-}*, *Tgfb β 2^{ΔB}* and *Iga^{-/-}* mice (n=6-8) that were re-stimulated *in vitro* with PMA/ionomycin and the representative flow cytometry panels (**e**). **f,g**, Flow cytometry of splenocytes from WT and *Tgfb β 2^{ΔB}* for: CD19⁺IgM⁺ cells (**f**) and IgA (**g**) gated on the splenic CD45⁺ population. **h-m**, Flow cytometry of splenocytes from WT, *Pd11/2^{-/-}* and *Il10^{-/-}* mice for: CD45⁺CD19⁺IgM⁺ cells (**h**), CD45⁺IgA⁺ cells (**i**), PD-L1 expression by CD19⁺IgM⁺ cells (**j**), and IL-10 expression by CD19⁺ cells (**k**). **l,m**, Serum IgA and IgG concentrations were analyzed in WT, *Pd11/2^{-/-}* and *Il10^{-/-}* mice (n=4-5 mice per group). All results are means $\pm$ s.e.m. Mann-Whitney and t tests were used to calculate statistical significance shown as *P , 0.05; **P , 0.01; ***P , 0.001. The different gating strategies and staining controls are shown. **n**, Gating strategies for analysis of splenocytes: lymphocyte gate, dead cell exclusion, doublets exclusion, and gating on the CD45⁺ population. **o**, Gating strategies for tumor-infiltrating lymphocytes: lymphocyte gate, dead cell exclusion, doublets exclusion, and gating on the CD45⁺ population. **p**, Flow cytometric analysis of IL-10 and IgA expression, gated on the CD45⁺ population: 1) isotype control (no staining), 2) non-stimulated splenocytes: showing IgA staining, but not IL-10. 3) stimulated splenocytes from *Il10^{-/-}* mice showing IgA staining, but not IL-10. 4) stimulated splenocytes from WT mice showing IgA and IL-10 staining. **q**, Flow cytometric analysis of IL-10 and CD19 expression, gated on the CD19⁺B220⁺

population. left: stimulated cells from *Il10*^{-/-} mice, showing B cell staining, but not IL-10; right: stimulated cells from WT mice showing B cell staining and IL-10 staining. **r**, Flow cytometric analysis of IL-10 and IgA expression, gated on the IgA⁺ population: left: stimulated cells from *Il10*^{-/-} mice, showing IgA cell staining, but not IL-10; right: stimulated cells from WT mice showing IgA and IL-10 staining. These results confirm IL-10 production by IgA⁺ cells. **s**, Flow cytometric analysis of p-STAT1 staining with corresponding isotype control.

Figure 16: Anti-PD-L1 treatment induce hepatocellular carcinoma tumor regression only when combined with oxaliplatin. WT mice bearing Hepatocellular carcinoma tumors (DEN-treated) were divided into four treatment groups. 1) isotype control (IgG2a); 2) oxaliplatin (weekly), 3) anti-PD-L1 (weekly); 4) oxaliplatin and anti-PD-L1 (weekly). After 4 treatment cycles, mice were sacrificed and analyzed. Tumor size are shown. Significance was determined by t tests.

Figure 17: Amino acid sequence of programmed cell death 1 ligand 1 isoform a precursor (PD-L1) Homo sapiens) A. NCBI Reference Sequence: NP_054862.1 (SEQ ID NO:01), B. Entrez Gene: 29126, NCBI Reference Sequence: NC_000009.12 (SEQ ID NO:02), C. Entrez Gene: 29126, NCBI Reference Sequence: NC_000009.12 (SEQ ID NO:03), and D. Entrez Gene: 29126, NCBI Reference Sequence: NC_000009.12 (SEQ ID NO:04).

Figure 18: Amino acid sequence of interleukin 10 (IL10) (Homo sapiens) NP_00563.1 ; Entrez Gene: 3586 (SEQ ID NO:05).

Figure 19: Amino acid sequence of portions of immunoglobulin A (IgA). **A.** IGHA1_HUMAN, P01876, Entrez Gene: 3493, NCBI Reference Sequence: NC_000014.9, containing Ig alpha-1 chain C region, Binding site P01876[352] (SEQ ID NO:06). **B.** IGHA2_HUMAN, Entrez Gene: 3494, P01877, containing Ig alpha-2 chain C region P01877 [1-340], PRO_0000153567 (SEQ ID NO:07).

DEFINITIONS

To facilitate understanding of the invention, a number of terms are defined below.

“Oxaliplatin” is a non-myeloablative or lymphodepleting chemotherapy drug. Exemplary dosage include 30-65mg/m² weekly or every two weeks, and may be adjusted to minimize lymphocyte and/or T cell number depletion. In one embodiment, the number of white blood cells is calculated weekly using, for example, a hemocytometer or flow cytometer. The dosage is decreased if the lymphocyte, and more particularly T cell, number decreases.

"TGF beta receptor ligand" is a molecule that binds to the TGF beta receptor, and is exemplified by Bone morphogenetic proteins (BMPs), Growth and differentiation factors (GDFs), Anti-müllerian hormone (AMH), Activin, Nodal, and TGFβ's.

5 "Treating" disease (e.g., cancer) refers to delaying, reducing, palliating, ameliorating, stabilizing, preventing and/or reversing one or more symptoms (such as objective, subjective, pathological, clinical, sub-clinical, etc.) of the disease. Objective symptoms are exemplified by tumor size (e.g. dimensions, weight and/or volume), tumor number, rate of change in tumor size and/or number, presence of metastasis, metastasis size (e.g. dimensions, weight and/or volume), metastasis number, and/or rate of change in metastasis size and/or number. Subjective symptoms are exemplified
10 by pain, fatigue, *etc.* Cancer symptoms may be assessed by, for example, biopsy and histology, and blood tests to determine relevant enzyme levels or circulating antigen or antibody, and imaging tests which can be used to detect a decrease in the growth rate or size of a neoplasm.

The terms "therapeutic amount," "pharmaceutically effective amount," and "therapeutically effective amount," are used interchangeably herein to refer to an amount that is sufficient to achieve a
15 desired result, such as treating disease.

"Cancer cell" refers to a cell undergoing early, intermediate or advanced stages of multi-step neoplastic progression as previously described (Pitot et al., Fundamentals of Oncology, 15-28 (1978)). This includes cells in early, intermediate and advanced stages of neoplastic progression including "pre-neoplastic cells (i.e., "hyperplastic cells and dysplastic cells), and neoplastic cells in advanced stages of
20 neoplastic progression of a dysplastic cell.

"Metastatic" cancer cell refers to a cancer cell that is translocated from a primary cancer site (*i.e.*, a location where the cancer cell initially formed from a normal, hyperplastic or dysplastic cell) to a site other than the primary site, where the translocated cancer cell lodges and proliferates.

"Cancer" refers to a plurality of cancer cells that may or may not be metastatic, such as prostate
25 cancer, liver cancer, bladder cancer, skin cancer (e.g., cutaneous, melanoma, basal cell carcinoma, Kaposi's sarcoma, etc.), ovarian cancer, breast cancer, lung cancer, cervical cancer, pancreatic cancer, colon cancer, stomach cancer, esophagus cancer, mouth cancer, tongue cancer, gum cancer, muscle cancer, heart cancer, bronchial cancer, testis cancer, kidney cancer, endometrium cancer, and uterus cancer. Cancer may be a primary cancer, recurrent cancer, and/or metastatic cancer. The place where a
30 cancer starts in the body is called the "primary cancer" or "primary site." If cancer cells spread to another part of the body the new area of cancer is called a "secondary cancer" or a "metastasis."

"Recurrent cancer" means the presence of cancer after treatment and after a period of time during which the cancer cannot be detected. The same cancer may be detected at the primary site or somewhere else in the body, e.g., as a metastasis.

"Epithelial cancer" refers to cancer that starts in epithelial tissues. Epithelial tissue covers the outside of the body as the skin, covers and lines all the organs inside the body, such as the organs of the digestive system, and lines the body cavities, such as the inside of the chest cavity and the abdominal cavity.

The term "specifically binds" and "specific binding" when made in reference to the binding of antibody to a target molecule (e.g., peptide) or to a target cell (e.g., immunosuppressive B cells), refer to an interaction of the antibody with one or more epitopes on the target molecule or target cell where the interaction is dependent upon the presence of a particular structure on the target molecule or target cell. For example, if an antibody is specific for epitope "A" on the target cell, then the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody. In one embodiment, the level of binding of an antibody to a target molecule or target cell is determined using the "IC₅₀" i.e., "half maximal inhibitory concentration" that refer to the concentration of a substance (e.g., inhibitor, antagonist, etc.) that produces a 50% inhibition of a given biological process, or a component of a process (e.g., an enzyme, antibody, cell, cell receptor, microorganism, etc.). It is commonly used as a measure of an antagonist substance's potency.

"Mammalian subject" includes human, non-human primate, murine (e.g., mouse, rat, guinea pig, hamster), ovine, bovine, ruminant, lagomorph, porcine, caprine, equine, canine, feline, ave, *etc.*).

A subject "in need" of treatment with the invention's methods includes a subject that is "suffering from disease," i.e., a subject that is experiencing and/or exhibiting one or more symptoms of the disease, and subject "at risk" of the disease. A subject "in need" of treatment includes animal models of the disease. Subject "at risk" of disease refers to a subject that is not currently exhibiting disease symptoms and is predisposed to expressing one or more symptoms of the disease. This predisposition may be genetic based on family history, genetic factors, environmental factors such as exposure to detrimental compounds present in the environment, *etc.*). It is not intended that the present invention be limited to any particular signs or symptoms. Thus, it is intended that the present invention encompass subjects that are experiencing any range of disease, from sub-clinical symptoms

to full-blown disease, wherein the subject exhibits at least one of the indicia (*e.g.*, signs and symptoms) associated with the disease.

“Immunosuppressive B cells,” “immunosuppressive plasmacyte cells,” “immunosuppressive plasma cells,” interchangeably refer to B lymphocyte cells that impede T-cell-dependent immunogenic chemotherapy (see examples 2-6) and are characterized by expressing PD-L1 (exemplified in Figure 17) and Interleukin-10 (exemplified in Figure 18) (IL10⁺ PD-L1⁺). In some embodiments, immunosuppressive B cells further express immunoglobulin A (IgA⁺ IL10⁺ PD-L1⁺) (Figure 19). In one embodiment, immunosuppressive B cells are distinct from the LT-producing CD20⁺ B cells.

“Immunogenic cell death” (“ICD”) is a form of cell death caused by some cytostatic agents such as oxaliplatin, cyclophosphamide, and mitoxantrone (Galluzzi et al., Cancer Cell, 2015 Dec 14;28(6):690-714) and anthracyclines, bortezomib, radiotherapy and photodynamic therapy (PDT) (Garg et al. (2010) “Immunogenic cell death, DAMPs and anticancer therapeutics: an emerging amalgamation.”. Biochim Biophys Acta 1805 (1): 53–71). Unlike normal apoptosis, which is mostly nonimmunogenic or even tolerogenic, immunogenic apoptosis of cancer cells can induce an effective antitumour immune response through activation of dendritic cells (DCs) and consequent activation of specific T cell response. ICD is characterized by secretion of damage-associated molecular patterns (DAMPs).

The terms “programmed cell death 1 ligand 1 isoform a precursor” and PD-L1” (also known as CD274; B7-H; B7H1; PDL1; PD-L1; PDCD1L1; PDCD1LG1) refer to the immune inhibitory receptor ligand that is expressed by hematopoietic and non-hematopoietic cells, such as T cells and B cells and various types of tumor cells. The encoded protein is a type I transmembrane protein that has immunoglobulin V-like and C-like domains. Interaction of this ligand with its receptor inhibits T-cell activation and cytokine production. During infection or inflammation of normal tissue, this interaction is important for preventing autoimmunity by maintaining homeostasis of the immune response. In tumor microenvironments, this interaction provides an immune escape for tumor cells through cytotoxic T-cell inactivation. Expression of this gene in tumor cells is considered to be prognostic in many types of human malignancies, including colon cancer and renal cell carcinoma. Alternative splicing results in multiple transcript variants. Human PD-L1 amino acid sequence is exemplified by SEQ ID NO:01-04 (Figure 17).

The terms “interleukin 10” and “IL-10” (also known as CSIF; TGIF; GVHDS; IL10A) refer to a cytokine produced primarily by monocytes and to a lesser extent by lymphocytes. This cytokine has

pleiotropic effects in immunoregulation and inflammation. It down-regulates the expression of Th1 cytokines, MHC class II Ags, and costimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. Human interleukin 10 amino acid sequence is exemplified by SEQ ID NO:05 (Figure 18).

5 The terms "immunoglobulin A," "IgA," and "Ig alpha" refer to the major immunoglobulin class in body secretions. It may serve both to defend against local infection and to prevent access of foreign antigens to the general immunologic system. Portions of human IgA amino acid sequences are exemplified by SEQ ID NO:06-07 (Figure 19).

10 The terms "reduce," "inhibit," "diminish," "suppress," "decrease," and grammatical equivalents when in reference to the level of any molecule (*e.g.*, amino acid sequence, and nucleic acid sequence, antibody, etc.), cell (*e.g.*, B cell, T cell, tumor cell), and/or phenomenon (*e.g.*, disease symptom), in a first sample (or in a first subject) relative to a second sample (or relative to a second subject), mean that the quantity of molecule, cell and/or phenomenon in the first sample (or in the first subject) is lower than in the second sample (or in the second subject) by any amount that is statistically significant
15 using any art-accepted statistical method of analysis.

20 The terms "increase," "elevate," "raise," and grammatical equivalents (including "higher," "greater," *etc.*) when in reference to the level of any molecule (*e.g.*, amino acid sequence, and nucleic acid sequence, antibody, etc.), cell (*e.g.*, B cell, T cell, tumor cell), and/or phenomenon (*e.g.*, disease symptom), in a first sample (or in a first subject) relative to a second sample (or relative to a second subject), mean that the quantity of the molecule, cell and/or phenomenon in the first sample (or in the first subject) is higher than in the second sample (or in the second subject) by any amount that is statistically significant using any art-accepted statistical method of analysis.

DESCRIPTION OF THE INVENTION

25 The invention provides a method for treating cancer in a subject in need thereof, wherein said subject comprises cancer tissue that contains epithelial cancer cells and immunosuppressive B cells, and wherein said method comprises administering to said subject a therapeutically effective amount of a) one or more first composition that causes immunogenic cell death and/or of said epithelial cancer cells, and b) one or more second composition that reduces one or both of the number and function of
30 said immunosuppressive B cells in said cancer.

Data herein show that successful eradication of large tumors in prostate (Examples 1-7) and liver (Example 8) by immunogenic chemotherapy requires removal of immunosuppressive plasmacytes that are present both in mouse and human PC. Three different, spontaneous and transplantable, mouse models of PC contain IgA⁺ plasmacytes that strongly suppress activation of CD8⁺ TIL after treatment of tumor-bearing mice with oxaliplatin. Unlike cisplatin, oxaliplatin induces immunogenic cell death that augments CTL activation and consequent tumor eradication^{5,28}. Although oxaliplatin causes regression of small tumors (< 100 mm³), it does not induce CTL activation and eradication of large (> 400 mm³) prostate tumors, despite induction of a DNA damage response. The main impediment to successful eradication of large tumors are tumor-infiltrating immunosuppressive B cells, that express IgA and CD138 but have down-regulated CD20 expression and therefore exhibit a typical plasmacyte phenotype. Nearly all of these cells express PD-L1 and about half of them also express IL-10 and Fas-L. Importantly, genetic analysis confirms that much of the immunosuppressive activity is derived from IgA⁺PD-L1⁺IL-10⁺ cells. Development of these cells, which are distinct from the LT-producing CD20⁺ B cells that infiltrate androgen-deprived prostate tumors and stimulate CRPC emergence through the IKK α -Bmi1 module^{7,8}, depends on TGF β R signaling. Nonetheless, the IgA⁺ plasmacytes may have evolved from CD20⁺LT⁺ B cells upon encounter of high TGF β concentrations and antigen in the PC microenvironment after oxaliplatin treatment. Ablation of T cell TGF β 1 was reported to augment CTL activation and inhibit tumor growth in *TRAMP* mice³⁹, and some of the IgA⁺ plasmacytes in human PC are located next to T cells. Another important TGF β source are the α SMA⁺ myofibroblasts that also reside next to IgA⁺ cells in oxaliplatin-treated mouse tumors and human PC samples¹⁶. Alternatively, LT-producing B cells may also stimulate the IgA CSR, as signaling via LT β receptor on gut *lamina propria* stromal cells is required for IgA production⁴⁰. Although the anti-inflammatory activity of intestinal IgA-producing cells is well known⁴¹, this is the first time IgA⁺ plasmacytes were found to suppress anti-tumor immunity.

As long as numerous IgA⁺ plasmacytes are present within prostate tumors, oxaliplatin fails to evoke a productive CTL-dependent anti-tumor response. Instead, CD8⁺ TIL express markers of exhaustion, including PD-1, TIM-3 and BTLA³. Suppression of the anti-tumor CTL response by IgA⁺ plasma cells requires PD-L1 and IL-10, both of which can induce anergy or exhaustion^{3,37}. Suppression may also involve Fas-L which can kill activated TILs⁴². B cell-mediated immunosuppression is also seen in adoptive transfer experiments, where B cells block the ability of transferred T cells to fully eradicate large prostate tumors in oxaliplatin-treated *TRAMP* mice or in mice bearing TRAMP-C2 or

MC tumors. Immunosuppression requires TGF β R2 expression on B cells, suggesting it is mediated by IgA⁺ plasmocytes. Yet, other mechanisms by which B cells regulate anti-tumor immunity were previously proposed^{43,44}, including indirect control of T cell infiltration via macrophages²¹ and IL-10 production by Breg cells⁴⁵. However, Breg-mediated suppression was only found to affect CD4⁺ T helper cells^{43,44,46}. Notably, IL-10-expressing IgA⁺ cells are most abundant in therapy-resistant and metastatic human PC and circulating IgA is a well-established adverse prognostic indicator in PC⁴⁷.

Data herein demonstrate that elimination or inhibition of tumor infiltrating IgA⁺ plasmocytes is important to successful immunotherapy of cancer (such as PC, liver cancer, *etc.*) as long as an immunogenic chemotherapeutic, such as oxaliplatin, is also used. Data herein also show that immunogenic chemotherapy enhances response rates to PD-1 or PD-L1 blockade in other malignancies, including bladder cancer and cutaneous melanoma where up to 35% of the patients were found to be responsive⁴⁸⁻⁵⁰.

The invention's methods use a first composition that induces immunogenic cell death (ICD), and that is referred to as "immunogenic chemotherapeutic compound" or "chemoimmunotherapeutic compound." This first composition is exemplified by oxaliplatin, cyclophosphamide, and mitoxantrone (Galluzzi et al., Cancer Cell. 2015 Dec 14;28(6):690-714) and anthracyclines, bortezomib, radiotherapy and photodynamic therapy (PDT) (Garg et al. (2010)"Immunogenic cell death, DAMPs and anticancer therapeutics: an emerging amalgamation." Biochim Biophys Acta 1805 (1): 53-71). doi:10.1016/j.bbcan.2009.08.003. PMID 19720113)

In one preferred embodiment, the compound that causes immunogenic cell death comprises oxaliplatin.

The invention's methods use a second composition, in combination with the above-described first composition, wherein the second composition reduces one or both of the number and function of the immunosuppressive plasmocyte cells in the cancer that is being treated. In one embodiment, the second composition that reduces one or both of the number and function of the immunosuppressive plasmocyte cells comprises antibody that specifically binds to said immunosuppressive B cells. This is illustrated by the use of a combination of anti-CD19 antibody (Int J Mol Cell Med. 2015 Summer;4(3):143-51 Anti-CD19 Monoclonal Antibodies: a New Approach to Lymphoma Therapy. Naddafi F, Davami) and anti-CD20 antibody (rituximab). Exemplary antibody dosage is 375 mg/m² intravenous infusion on day 1 of the first of 4 to 5 cycles.

In one embodiment, the second composition that reduces one or both of the number and function of the immunosuppressive plasmocyte cells comprises anti-PDL1 antibody (also referred to as “anti-PD-L1 antibody” and “aPDL1”). Exemplary anti-PDL1 antibodies that reduce the function, development, and/or number of PD-L1, are commercially available (Genentech) and are in clinical trials (See, A Phase 1 Study of Atezolizumab (an Engineered Anti-PDL1 Antibody) in Patients with Locally Advanced or Metastatic Solid Tumors. ClinicalTrials.gov Identifier: NCT01375842).

In one embodiment, the second composition that reduces one or both of the number and function of the immunosuppressive plasmocyte cells comprises “anti-CD19-CAR T cells,” which are chimeric Antigen Receptor T Cells against CD19 for Multiple Myeloma, described in Garfall et al., N Engl J Med, 2015 Sep 10;373(11):1040-7.

In one embodiment, the immunosuppressive B cells comprise TGF beta receptors, and the second composition that reduces one or both of the number and function of said immunosuppressive plasmocyte cells in the cancer being treated is a compound that inhibits binding of said TGF beta receptor to a TGF beta receptor ligand, and is exemplified by the small molecule “ALK5 inhibitor,” which is in clinical trials (Herbertz et al., Drug Des Devel Ther. 2015 Aug 10;9:4479-99. doi: 10.2147/DDDT.S86621. eCollection 2015).

The term “administering” to a subject means delivering a molecule to a subject. “Administering” a composition to a subject in need of reducing a disease and/or of reducing one or more disease symptoms includes prophylactic administration of the composition (i.e., before the disease and/or one or more symptoms of the disease are detectable) and/or therapeutic administration of the composition (i.e., after the disease and/or one or more symptoms of the disease are detectable). The invention’s methods include administering a combination of a first composition that causes immunogenic cell death, and a second composition that reduces one or both of the number and function of immunosuppressive plasmocyte cells. The first and second compositions may be administered simultaneously at substantially the same time, and/or administered sequentially at different times in any order (first composition followed second composition, or second composition followed by first composition). For example, administering said second composition substantially simultaneously and sequentially in any order includes, for example, (a) administering the first and second compositions simultaneously at substantially the same time, followed by administering the first composition then the second composition at different times, (b) administering the first and second compositions simultaneously at substantially the same time, followed by administering the second

composition then the first composition at different times, (c) administering the first composition then the second composition at different times, followed by administering the first and second compositions simultaneously at substantially the same time, and (d) administering the second composition then the first composition at different times, followed by administering the first and second compositions simultaneously at substantially the same time.

Administering may be done using methods known in the art (e.g., Erickson et al., U.S. Patent 6,632,979; Furuta et al., U.S. Patent 6,905,839; Jackobsen et al., U.S. Patent 6,238,878; Simon et al., U.S. Patent 5,851,789). The invention's compositions may be administered prophylactically (*i.e.*, before the observation of disease symptoms) and/or therapeutically (*i.e.*, after the observation of disease symptoms). Administration also may be concomitant with (*i.e.*, at the same time as, or during) manifestation of one or more disease symptoms. Also, the invention's compositions may be administered before, concomitantly with, and/or after administration of another type of drug or therapeutic procedure (e.g., surgery).

Methods of administering the invention's compositions include, without limitation, administration in parenteral, oral, intraperitoneal, intranasal, topical and sublingual forms. Parenteral routes of administration include, for example, subcutaneous, intravenous, intramuscular, intrasternal injection, and infusion routes. In a preferred embodiment, administration in intraperitoneal and/or intravenous.

Antibody treatment of human beings with cancer is known in the art, for example in U.S. Pat. Nos. 5,736,137; 6,333,410; 5,475,092; 5,585,499; 5,846,545; 7,202,346; 6,340,701; 6,372,738; 7,202,346; 5,846,545; 5,585,499; 5,475,092; 7,202,346; 7,662,387; 7,662,387; 6,429,295; 7,666,425; 5,057,313.

The invention's antibodies may be administered with pharmaceutically acceptable carriers, diluents, and/or excipients. Examples of suitable carriers, diluents and/or excipients include: (1) Dulbecco's phosphate buffered saline, pH about 7.4, containing about 1 mg/ml to 25 mg/ml human serum albumin, (2) 0.9% saline (0.9% w/v NaCl), and (3) 5% (w/v) dextrose.

A) Prostate Cancer

Cancer development and response to therapy are strongly influenced by innate and adaptive immunity. For example, androgen depletion and malignant progression affect recruitment of lymphocytes into prostate tumors, including B cells that produce lymphotoxin and thereby stimulate expansion of androgen-deprived prostate cancer (PC) progenitors. Normally prostate cancer does not

respond to an immunogenic (low) dose of oxaliplatin. The invention's methods sensitize prostate cancer to low-dose oxaliplatin and achieve nearly complete tumor elimination through activation of cytotoxic T cells. The invention's method is based on removal or inactivation of immunosuppressive B cells that accumulate in prostate tumors and prevent the activation of cytotoxic T cells.

5 Aggressive prostate cancer is refractive to chemotherapy and so far attempts to treat prostate cancer with immune checkpoint inhibitors have failed. Data herein (Examples 1-7) show that prostate cancer does not respond to low immunosuppressive doses of oxaliplatin, a chemotherapeutic drug, due to accumulation of immunosuppressive B cells that prevent the activation of cytotoxic T cells. By getting rid of those B cells we were able to fully sensitize prostate tumors to low doses of oxaliplatin and achieve nearly complete tumor elimination. Immunosuppressive B cells are present in both mouse
10 and human prostate cancer.

Data herein (Examples 1-7) show that B cells also determine how PC responds to the immunogenic chemotherapeutic agent oxaliplatin, which at a low, non-myelosuppressive, dose induces immunogenic cell death that can promote activation of tumor-directed cytotoxic T cells (CTL).
15 However, three different mouse models of PC do not respond to oxaliplatin unless genetically or pharmacologically depleted of B cells. The B lymphocytes found to suppress CTL activation are plasmacytes that express IgA, IL-10 and PD-L1, whose appearance depends on TGF β -receptor (TGF β R) signaling. Elimination of these cells, which also infiltrate human therapy-resistant PC, allows CTL-dependent eradication of oxaliplatin-treated tumors.

20 Currently, there are immune checkpoint therapies based on inhibition of CTLA4, PD-1 or PD-L1. Also, there are B cell targeting drugs such as Rituxan. However, immunosuppressive B cells are completely refractory to these drugs.

Data herein (Examples 1-7) confirmed the efficacy of the invention's method in two mouse models of prostate cancer, and also confirmed the presence of immunosuppressive B cells in human
25 prostate cancer. Since CRPC is refractory to most therapies^{9,10}, data herein in Examples 1-7 examined the role of B cells in acquisition of chemotherapy resistance. We focused our studies on oxaliplatin, an immunogenic chemotherapeutic drug^{4,5} that is effective in aggressive PC^{9,10}.

B) Liver cancer:

Example 8 shows that immunosuppressive B cells are present in hepatocellular carcinoma tumors, and the combination therapy of oxaliplatin with checkpoint inhibitor (aPD-L1) decreased the number of liver tumors.

5 EXPERIMENTAL

The following serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof. See Shalapour et al., Nature (2015) 521(7550):94-8. doi: 10.1038/nature14395.

10 EXAMPLE 1: Materials and Methods

All *TRAMP* mice, their derivatives and s.c. Myc-Cap transplantation were in the FVB genetic background and their basic immune parameters are shown in Fig. 15a-m. For s.c. TRAMP-C2 tumors, C57BL/6 mice were used. In both s.c. tumors, $2-3 \times 10^6$ cells/mouse were transplanted. More detailed information is as follows.

15 Animal models

C57BL/6 and *FVB* control mice were purchased from Charles River Laboratories and *CD45.1* mice¹ were purchased from Jackson Laboratory, and all were bred at the University of California San Diego (UCSD) animal facility. *C57BL/6-Tg(TRAMP)8247Ng/J* (*TRAMP* mice)² were backcrossed to the *FVB* strain for more than 10 generations. The median survival of *TRAMP-FVB* mice was 23 weeks compared to 52 weeks for *TRAMP-C57BL/6* mice. *TRAMP* mice were crossed with B cell-deficient (*Jh*^{-/-}) mice³, CTL-deficient (*Cd8a*^{-/-}) mice⁴ or *Rag1*^{-/-} mice, which lack both B and T cells⁵, all in the FVB-background. *OT-I* mice were obtained from Taconic⁶. *Tgfb β 2*^{F/F} (*FVB*-background) mice were obtained from Dr. Hal Moses at Vanderbilt University⁷. *Tcrb*^{-/-}, *Cd19-Cre*, *Il10*^{-/-} and *CD45.1* mice were purchased from the Jackson Laboratory. IgA gene-deficient (*Iga*^{-/-}) mice⁸ were obtained from Baylor College of Medicine. *Pdl1*^{2-/-} mice were obtained from Genentech (San Francisco, CA). *Cd19-Cre* and *Iga*^{-/-} mice were backcrossed to the *FVB* strain for more than 10 generations. All mice were maintained in filter-topped cages on autoclaved food and water at the UCSD animal facility and all experiments were performed in accordance with UCSD and NIH guidelines and regulations.

25 The number of mice used in each experiment and the number of experiments are shown in Table 1 below.

Table 1

Experimental genotype	control	oxali (cispl)	number of experiments
MC-WT	22	24 (+6 cispl)	6
TR-WT	32	21	6
MC- <i>Jh</i> ^{-/-}	18	14 (+4 cispl)	4
TR- <i>Jh</i> ^{-/-}	19	18	4
MC- <i>Cd8a</i> ^{-/-}	10	11	2
TR- <i>Cd8a</i> ^{-/-}	19	18	3
MC- <i>Rag</i> ^{-/-}	9	10	2
TR- <i>Rag</i> ^{-/-}	9	9	2
MC- <i>Tgfb2</i> ^{AB}	7	9	3
MC- <i>Iga</i> ^{-/-}	8	9	3
MC- <i>Jh</i> ^{-/-} + B cell transfer	-	14	2
MC-WT + aPD-L1	10	10	2
TRAMP-C2 model	10	10	3
TRAMP-C2 model T cell transfer	8	8	2
FVB control	17	13	2
MC- <i>Rag</i> ^{-/-} T cell + B cell transfer	5	18	2
MC-WT + B cell depletion	14	16	1
MC- <i>Cd8a</i> ^{-/-} + B cell depletion	6	6	1
Total	220	243	42
Total number: 468			

Mouse treatment studies were “matched design control trials.” Accordingly, mice were randomly chosen and paired based on sex (male), age (Fig. 6a) and tumor size. For transplanted tumor models, tumor size was defined by the median tumor volume (e.g. 400 mm³, for late treatments, Fig. 6a,f). For TRAMP transgenic tumor models, treatment decisions were made based on age and mice were randomly chosen including a control littermate. An identification code was assigned to each tumor-bearing mouse both in the transplanted and transgenic models, and the investigators were blinded to treatment allocation at the time of tumor volume measurement, autopsy and analysis.

Flow cytometry and lymphocyte isolation

For lymphocytes isolated from spleen and lymph nodes, standard protocols using filters have been used. Lymphocytes were isolated from human blood using Ficoll-Paque PLUS (GE Healthcare Life Science) according to manufacturer’s recommendations. For lymphocyte isolation from tumors (mouse and human), tumors were cut into small pieces and incubated in dissociation solution (RPMI medium supplemented with 5% FBS, collagenase type I (200 U/ml), collagenase type IV (200 U/ml),

and DNase I (100 µg/ml) for 30 min at 37° C. After incubation, cell suspensions were passed through a 50 µm cell strainer and washed twice. For large tumors (≥ 0.7 g), hematopoietic cells were pre-enriched using density gradient centrifugation (Percoll or Ficoll), and red blood cells were lysed (RBC Lysis buffer, multi-species; eBioscience). For blocking of Fc-mediated interactions, mouse cells were pre-incubated with 0.5-1 µg of purified anti-mouse CD16/CD32 (93) per 100 µl and human cells were incubated with FcR blocking reagent (Miltenyi Biotec). Isolated cells were stained with labeled antibodies in PBS with 2% FCS and 2 mM EDTA or cell staining buffer (Biolegend). Dead cells were excluded based on staining with Live/Dead fixable dye (eBioscience). For intracellular cytokine staining, cells were restimulated (Myc-Cap cell lysate, PMA/ionomycin or PMA/ionomycin/LPS, as indicated) in the presence of a protein transport inhibitor cocktail containing Brefeldin A and Monensin (eBioscience), as indicated. For CD107, a staining antibody was added to the culture during the stimulation. After 5 hrs, cells were fixed and permeabilized with BD™ Cytofix/Cytoperm reagent for cytokine staining. BD™ transcription factor buffer was used for Foxp3 and T-bet staining and BD™ Phosflow was used for p-SMAD2/3 and p-STAT staining (BD Biosciences) according to manufacturer's recommendations. After fixation/permeabilization, cells were stained with labeled antibodies of interest. Moreover, *Il10* and *β-actin* mRNA expression were analyzed on single cell level by flow cytometry in combination with CD45, IgA and IL-10 protein staining, using FlowRNA II Assay kit (Affymetrix eBioscience) according to manufacturer's protocols⁹. Cells were analyzed on a Beckman Coulter Cyan ADP flow cytometer. Data were analyzed using FlowJo software (Treestar). Immune cell analysis of tumor-free mice of different genetic backgrounds (*C57BL/6* and *FVB*) and different genetic ablations are shown in Fig. 15a-m. The gating strategies and isotype controls for p-STAT1 and IL-10 staining are shown in Fig. 15n-q.

Adoptive lymphocyte transfer

For adoptive T cell transfer (ATCT) CD8⁺ T cells were isolated from single cell suspensions, prepared from spleens and lymph nodes as described above, using CD8α-specific microbeads and MACS-columns (both Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), and 5×10^6 CD8⁺ T cells were transferred intraperitoneally (i.p.; Fig. 14a-j). For adoptive B cell transfer (ABCT), B cells (B220⁺/CD19⁺) were isolated from single cell suspensions prepared from spleens using CD19- and B220-specific microbeads and MACS-columns, and 5×10^6 B cells were transferred i.p. (Fig. 4g-h, Fig. 12n,o). For adoptive splenocytes transfer (ACT), single cell suspensions prepared from spleens

were transferred i.p., with one total spleen injected per mouse. Labelling with 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) was done according to manufacturer's protocol. 5×10^6 CD8⁺ T cells or 7×10^6 B cells were transferred (equal to one spleen per mouse; Fig. 5a-i and Fig. 14k-q). For combined adoptive B and T cell transfer (Fig. 5j-l, Fig. 14r-t), T cells were isolated from WT-FVB mice immunized with a Myc-Cap cell lysate as previously described¹⁰. Specifically, Myc-Cap cells were incubated *in vitro* with oxaliplatin (40 μ M) for 48 hrs. The extent of cell death was determined by flow cytometry, showing that more than 90% of cells were positive for Annexin V and PI. The dead cells were injected subcutaneously (s.c.) into WT-FVB mice. Seven days later, T cells were isolated from single cell suspensions of spleen and LN using a Pan T cell isolation Kit (Miltenyi Biotec). B cells were isolated from spleens of naïve FVB-WT or FVB-*Tgfb β 2^{ΔB}* mice using a Pan B cell isolation kit (Miltenyi Biotec). MC-tumor bearing *Rag1*^{-/-} mice received 5×10^6 T cells with or without 5×10^6 B cells (98% pure) from WT or *Tgfb β 2^{ΔB}* mice. Purity was analyzed on a Beckman Coulter Cyan ADP flow cytometer and was always > 98%.

Absolute numbers of particular immune cells in spleen were calculated by multiplying the CD45⁺ cell number from one spleen by the percentages of the particular cell type amongst CD45⁺ cells. Absolute numbers of particular immune cells (e.g. CD8⁺ cells) in tumors was calculated by multiplying the cell number in one tumor portion by the percentages of the corresponding cell type in vital tumor cells divided by the weight of the analyzed tumor fragment.

20 Subcutaneous tumor models

2×10^6 Myc-Cap¹¹ or 3×10^6 TRAMP-C2¹² cells (purchased from ATCC) were s.c. injected into the right flank. Tumors were measured every 2-3 days using a caliper. Tumor volumes were calculated as $\text{width}^2 \times \text{length} / 2$.

25 Immune-mediated B cell depletion

B cells were depleted as previously described¹³. Mice were weekly injected (i.p.) with a mixture of monoclonal antibodies, each at 150 μ g/mouse: rat anti-mouse CD19 (clone 1D3), rat anti-mouse B220 (clone RA36B2), and mouse anti-mouse CD22 (clone CY34). After 48 hrs, the mice were injected with a secondary antibody (mouse anti-rat kappa chain; GeneTex) at 150 μ g/mouse. In addition, mice were injected weekly, but not on the same day, with 250 μ g/mouse rat anti-mouse CD20 (Genentech). Rat anti-mouse IgG2a and IgG1 were used as isotype controls. Mice were treated for 3

weeks in total (Fig. 1g; Fig. 7i-p).

Oncomine data analysis

In silico analysis of human IgA (*IGHA1*) mRNA expression was performed using 15 PC microarray gene datasets¹⁴⁻²⁸ from the Oncomine database (Compendia Biosciences; Ann Arbor, MI, USA; www.oncomine.org)²⁹ comparing a combined 126 carcinoma/adenocarcinoma specimens to 30 normal (either benign, disease-free normal and/or normal adjacent) tissue specimens. Evaluation criteria were set as $p < 0.05$, fold change > 2.0 , and gene rank in the top 10%.

Analysis of human specimens

Paraffin-embedded specimens from a total of 110 PC patients were integrated into a tissue microarray system (TMA) constructed at the Clinical Institute of Pathology at the Medical University of Vienna (MUV). All of the human specimens used for TMA construction were approved by the MUV Research Ethics Committee (1753/2014). The cohort included 87 patients with early PC (E-PC), 9 patients with therapy-resistant PC (TR-PC), and 15 patients with metastatic PC (M-PC). Patients' demographic and histopathological features are shown below in Table 2.

Table 2

		Ratio CD20+/CD8+ (IRA%) ^b			IgA-CD138+ double positive cases	
		N	Median (1 st -3 rd quartile)	P ^c	%	P ^d
All cases		110	0.25 (0.06-1.00)		41.8	
Patient demographics						
Age	< 75 yrs.	43	0.20 (0.10-0.36)	0.49	37.2	0.16
	≥ 75 yrs.	43	0.13 (0.07-0.75)		23.3	
	NA	24				
Tumor features						
pT staging	pT2	56	0.23 (0.10-0.47)	0.48	35.7	0.81
	pT3/pT4	36	0.15 (0.06-0.66)		33.3	
	NA ^a	18				
Gleason grade	≤6	36	0.23 (0.11-1.03)	0.10	27.8	0.23
	7	43	0.12 (0.07-0.35)		32.6	
	≥8	13	0.34 (0.19-0.75)		53.8	

	NA ^a	18				
Stage of disease	Early stage	86	0.20 (0.07-0.51)		30.2	
	Therapy resistance	9	0.67 (0.34-1.35)	<0.001	77.8	<0.001
	Metastasis	15	2.63 (0.33-8.44)		86.7	

^aNot available (NA) cases were not included in the analysis.

^b Ratio CD20⁺/CD8⁺ cells (IRA%) is expressed as percentage immunoreactive area

^c By Mann-Whitney test.

^d By Chi-square test.

5

TMA were designed to provide two cores of normal prostate tissue and four cores of PC tissue from each E-PC patient, and 3-6 cores of tumor tissue for each TR-PC and M-PC patient. Stained TMA slides were digitalized by virtual microscopy at 20 × magnification with a fixed light intensity and resolution into a bright-field image using the Nanozoomer (Hamamatsu) scanner. Computer-assisted image analysis of individual TMA core images was used to quantify the percentage of CD8⁺ and CD20⁺ immune reactive area (IRA%) as a proportion of the total digitized haematoxylin-stained region, as previously described³⁰. For each PC patient, the mean continuous values of CD8⁺ and CD20⁺ IRA% in TMA cores without technical artifact for normal and tumor prostate tissue were calculated and used for subsequent statistical analysis. The presence of CD138⁺ and IgA⁺ double immunoreactivity for plasma cells in the stromal compartment or directly contacting a cancer cell was semi-quantitatively scored in TMA cores for each patient by an investigator who was blinded to the patients tumor features. A value of 0 was assigned to tissue cores without evidence of stromal CD138⁺/IgA⁺ double immunoreactive cells and a value of 1 was recorded when CD138⁺/IgA⁺ double immunoreactive cells were present in the stromal compartment. Furthermore, after approval from the UCSD institutional review board (IRB), whole tissue slides were subjected to immunohistochemical (IHC) analysis of αSMA⁺/IgA, CD8⁺/IgA and IL-10⁺/IgA double staining from a cohort of formalin-fixed, paraffin-embedded (FFPE) radical prostatectomy specimens. As previously described³¹, this cohort included up to 50 patients, which were selected based on known clinical outcome according to risk categories of low-, intermediate- and high-risk groups based on the D'Amico risk classification³².

25 Anonymized fresh prostatectomy and blood samples from consented human subjects, and de-identified clinical information were provided under the UCSD Moores Cancer Center Biorepository and Tissue Technology IRB approved protocol and provided to investigators (M.K., C.J.K., C.A.M.J., D.E.H.) with Cancer Sample Banking Committee approval. Fresh, de-identified samples of human prostate tissue and blood in 10 ml EDTA-coated tubes were collected from patients undergoing radical

prostatectomy for clinically localized, intermediate or high risk PC, Gleason grade 3+4 or higher. A board-certified genitourinary pathologist (D.E.H.) collected samples of fresh prostate tumor and adjacent benign tissue, within 1 hr of radical prostatectomy, that were 5-10 mm in diameter.

5 Immunostaining

Tissues were embedded in Tissue Tek OCT (Sakura Finetek, Torrance, CA, USA) compound and snap-frozen. Tissue sections were fixed in cold acetone/methanol or 3% PFA for 3-10 min and washed with PBS. Slides were blocked with 1x PBS/1% normal donkey or goat serum for surface staining or 0.2% gelatin (from cold water fish skin; Sigma-Aldrich) /PBS/1% normal donkey or goat serum for intracellular staining for 30 min. Sections were incubated with primary antibodies for 1 or 12 hrs at RT or 4° C, respectively. After washing with PBS, secondary antibodies were added for 1 hr at RT. As negative controls, samples were incubated with isotype-matched control antibodies or secondary antibodies only. After staining with DAPI, sections were covered with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA USA). TMA tissue slides from formalin-processed and paraffin-embedded tumor sections were processed for immunohistochemistry. After deparaffinization and rehydration, sections were immersed in a pre-heated antigen retrieval water bath with a pH 6.1 citrate buffer, or Dako Target Retrieval Solution for 20 minutes at 95-96°C. ImmPRESS™ Polymer System Diaminobenzidine tetrahydrochloride (DAB) peroxidase substrate-based chromogens were used for single staining of CD8, CD20 and for IgA staining when combined with CD138 and for αSMA staining when combined with IgA for IHC of human samples. ImmPACT™ Vector® NovaRED™ peroxidase substrate-based chromogens were used for CD138 staining when combined with IgA for IHC of human samples. ImmPACT™ Vector® Red Alkanine Phosphatase substrate-based chromogens were used for IgA staining when combined with αSMA for IHC of human samples. All stainings were done according to the manufacturer's protocols (Vector Laboratories). Nuclei were lightly counterstained with a freshly made haematoxylin solution then further washed in water and mounted. Sections were examined using an Axioplan 200 microscope with AxioVision Release 4.5 software (Zeiss, Jena, Germany) or TCS SPE Leica confocal microscope (Leica, Germany).

30 Antibodies

Antibodies specific for the following antigens were used: mAb rabbit to cleaved Caspase 3 (# 9661) or p- γ H2AX (Ser139; 20E3) (Cell Signaling Technology, Danvers, USA); pAb rabbit to: CD3 (Dako, IS503); α SMA (Dako); Tim-3 (B8.2C12); Tim-1 (RMT1-4); p-SMAD2/3 (D27F4); LC3B (D11) and CD138 (Syndecan-1) (anti-mouse Biolegend; anti-human Dako M115); IgA (mA-6E1, m11-44-2, mRMA-1, anti-mouse eBioscience/Biolegend; anti-human for IHC: Dako, A0262; for FACS: Miltenyi); AID (MAID-2); CD8a (m53-6.7, human DAKO, C8/144B); CD45 (hOKT4); CD20 (AISB12, hL26); CD44 (IM7); CD4(RM4-5); B220 (RA3-6B2); CD19 (m1D3, hHIB19); IgM (II/41); IgD (11-26c); TNF (MP6-XT22); IFN γ (XMG1.2); GrzB (NGZB); CD107a (eBio1D48); PD-1 (J43); PD-L1 (MIH5); FAS-L1 (MFL3); Ki67 (SolA15); IgG2a (m2a-15F8); IgG1 (M1-14D12); IL-10 (mJES5-16E3; hJES3-9D7; IHC: hIL-10; AF-217-NA); CD69 (H1.2F3); FoxP3 (FJK/16s); CD11c (N418); CD11b (M1/70); MHCII (M5/114.15.2); Gr-1 (1A8-l66g); F4/80 (BM8) and NK1.1 (NKR.P1C) (all from eBioscience); CD31 (PECAM-1, MEC 13.3); CD45 (m30-F11); p-STAT1 (pY701) and p-STAT3 (pY705) (BD Bioscience); and α SMA (anti-mouse ab5694; anti-human: DAKO, 1A4). The following Alexa 594-, Alexa 647-, Alexa 488-conjugated secondary antibodies were used: donkey anti-rat IgG, donkey anti-rabbit IgG, donkey anti-goat IgG and goat anti-rat IgG (Molecular Probes, Invitrogen).

ELISA

Anti-SV40 Tag immunoglobulin ELISA was performed as previously described³³. IgA and IgG ELISA kits were purchased from eBioscience, and used according to manufacturer's protocols. Tumoral single cell suspension has been prepared as described above in the flow cytometry section, and was washed 2-3 times with 1xPBS/2mMEDTA/2% FCS, to remove soluble IgA. Thereafter, about 3×10^6 cells / 24-well were plated in either 10% FCS/DMEM or Hybridoma medium (Life Technology). After 24 hrs, the supernatants were analysed for IgA content. Media without cells were used as controls.

Treatment with chemotherapy or antibodies

Oxaliplatin was diluted in 5% dextrose and i.p. injected weekly at 6 mg/kg as indicated. Anti-PD-L1 antibody was i.v. injected at 10 mg/kg once, followed by 5 mg/kg bi-weekly. Mice were treated for three weeks for a total of 7 doses/animal.

Q-RT-PCR analysis

Total RNA was extracted using an RNeasy Plus kit (Qiagen). RNA was reverse transcribed using an IScript kit (Biorad). Q-RT-PCR was performed using Ssofast EvaGreen supermix (Biorad) on a Biorad CFX96 machine. Primer sequences are listed below and generally were obtained from the NIH qPrimerDepot (<http://mouseprimerdepot.nci.nih.gov>). The relative expression levels of target genes were measured in triplicates and normalized against the level of RPL32 expression. Fold-difference (as relative mRNA expression) was calculated by the comparative CT method ($2^{(Ct(RPL32) - Ct(\text{gene of interest}))}$).

Table 3

Name	primer 1	primer 2
IFN γ	TGAACGCTACACACTGCATCT	GACTCCTTTTCCGCTTCCTGA
TNF	GGTCTGGGCCATAGAACTGA	CAGCCTCTTCTCATTCCCTGC
IL-10	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT
Perforin	TGGAGGTTTTTGTACCAGGC	TAGCCAATTTTGCAGCTGAG
TGF β 1	AAGTTGGCATGGTAGCCCTT	GGAGAGCCCTGGATACCAAC
NOS2	TCCAGGGATTCTGGAACATT	GAAGAAAACCCCTTGTGCTG
Arginase 1	TTTTTCCAGCAGACCAGCTT	CATGAGCTCCAAGCCAAAGT
Granzyme B	CTCTCGAATAAGGAAGCCCC	CTGACCTTGCTCTGGCCTC
RPL32	TTGTGAGCAATCTCAGCACA	GGGAGCAACAAGAAAACCAA
IL-21	CCC TTG TCT GTC TGG TAG TCA TCT T	GGA GGC GAT CTG GCC C
IL-12p35	GAGGACTGAAGATGTACCAG	CTATCTGTGTGAGGAGGGC
IL-12p40	GAC CCT GCC CAT TGA ACT GGC	CAA CGT TGC ATC CTA GGA TCG
PD-L1	TGC TGC ATA ATC AGC TAC GG	CCA CGG AAA TTC TCT GGT TG

Statistical analysis

Data are presented either averages $\pm$ S.E.M or median of continuous values and were analyzed by Students' t-test or Mann-Whitney-U, respectively, for comparison of two groups. Kruskal-Wallis test was used to compare three or more groups. Long-rank (Mantel-Cox) tests were used to compare survival curves. Fisher's exact Chi-square P values were used to calculate statistical significance of categorical values between groups. Two tailed p-values of ≤ 0.05 were considered significant. Unpaired t test-independent studies were used to determine the minimum sample sizes (StatsDirect Version 2.8.0). GraphPad PRISM software was used for statistical analyses.

EXAMPLE 2: B cells confer oxaliplatin resistance

Using the TRAMP model of spontaneous PC which gives rise to metastatic tumors with a neuroendocrine phenotype typical of aggressive, drug resistant human PC¹¹, we examined how B and CD8⁺ (CTL) lymphocytes affect the response to low dose (LD) oxaliplatin by crossing *TRAMP (FVB)* mice to either B cell (*Jh*^{-/-})^{7,12} or CTL (*Cd8a*^{-/-})¹³ deficient mice. Although early (≤ 0.2 g) tumors responded to oxaliplatin regardless of their B cell status (Fig. 6a,b), upon reaching ≥ 0.7 g, tumors in WT *TRAMP* mice became largely resistant to “late” chemotherapy (Fig. 1a). However, PC growth in B cell-deficient *TRAMP;Jh*^{-/-} mice remained oxaliplatin sensitive (Fig. 1a), although the B cell deficiency had little effect on tumor development and histology (Fig. 6b-e). By contrast, CD8⁺ cell deficiency modestly attenuated therapeutic responsiveness in small tumors, but barely impacted large, oxaliplatin-resistant, tumors (Fig. 1a; Fig. 6b). Similar results were obtained in a different model based on s.c. transplantation of Myc-Cap (MC) cells¹⁴ (Fig. 6f,g). Whereas small MC tumors (≤ 100 mm³) were chemotherapy responsive even in WT mice (Fig. 6g), large MC tumors (≥ 350 -400 mm³) shrank upon oxaliplatin treatment only in *Jh*^{-/-} mice (Fig. 1b-d). No response was observed in *Cd8a*^{-/-} mice. Oxaliplatin responsiveness was associated with enhanced caspase 3 activation (Fig. 1e,f), indicating more effective apoptosis. However, the tumoral DNA damage response measured by histone H2AX phosphorylation was similarly activated by oxaliplatin, regardless of host genotype (Fig. 6h,i). Oxaliplatin treatment enhanced tumor infiltration with CD45⁺ cells in WT and *Jh*^{-/-} mice (Fig. 1c), but induction of myofibroblast activation and CD31 infiltration was more pronounced in WT mice (Fig. 6j-l). LD oxaliplatin enhanced *TRAMP* mouse survival in a manner dependent on CTL and inhibitable by B cells (Fig. 6m-p). B cell immuno-depletion also enhanced oxaliplatin-induced tumor regression and the effect was CTL-dependent (Fig. 1g).

EXAMPLE 3: B cells suppress CTL activation

Enhanced oxaliplatin responsiveness in B cell-deficient mice reflected more effective CTL activation. Oxaliplatin stimulated CD8⁺ cell recruitment in both *TRAMP* and *TRAMP;Jh*^{-/-} mice, although many more tumoral CD8⁺ cells were found in the latter (Fig. 2a; Fig. 7a). The B cell deficiency also enhanced CD8⁺ cell recruitment into MC tumors after LD oxaliplatin treatment (Fig. 2b,c; Fig. 7b). CD4⁺ T cell recruitment was also enhanced by B cell absence (Fig. 7c,d). The B cell deficiency strongly enhanced induction of perforin, γ interferon (IFN γ) and TNF by oxaliplatin in MC

tumors, with TNF and IFN γ produced by CD8 $^{+}$ cells (Fig. 2d-f; Fig. 7e). Oxaliplatin-treated MC tumors of *Jh $^{-/-}$* mice also contained more CD8 $^{+}$ cells with activated STAT1 (Fig. 7f). *Jh $^{-/-}$* tumors and spleens also contained more proliferative CD8a $^{+}$ CD44 hi GrzB $^{+}$ Ki67 $^{+}$ cells before and especially after oxaliplatin treatment (Fig. 2g) and fewer “exhausted”³ tumoral CD8 $^{+}$ CD44 $^{+}$ PD-1 $^{+}$ Tim3 $^{+}$ and CD8 $^{+}$ BTLA hi cells (Fig. 2h,i; Fig. 7g), whose presence in WT tumors was elevated after oxaliplatin treatment. Similar results were observed upon analysis of “exhausted” splenic CD8 $^{+}$ cells (Fig. 7h). B cell immuno-depletion also enhanced tumoral CTL activation (Fig. 7i-p).

EXAMPLE 4: The immunosuppressive B cells are IgA $^{+}$ plasma cells

Which tumor infiltrating B cells suppress CTL activation? Oxaliplatin treatment greatly increased the number of tumoral B220 $^{+}$ CD19 $^{+}$ B cells (Fig. 3a, Fig. 8a,b). After 3-4 cycles of oxaliplatin treatment, at least 40% of the tumoral B cells were CD19 $^{+}$ B220 low CD138 $^{+}$ plasma cells, depending on the tumor model used, 40-80% of which expressed IgA (Fig. 3b,c; Fig. 8c-l). As described¹⁵, plasma cells down-regulated CD20 expression (Fig. 8c-l). IgA $^{+}$ B cells became detectable 48 hrs after the first oxaliplatin cycle, and their abundance increased to nearly 80% of B220 low cells after additional cycles (Fig. 8g,l). When cultured *ex vivo*, tumoral IgA $^{+}$ B cells released soluble IgA (Fig. 9a). Oxaliplatin treatment also increased serum IgA, but had little effect on serum IgG, in both TRAMP and MC-tumor models (Fig. 9b-e). IgA $^{+}$ cells with plasmocyte morphology were often found adjacent to α smooth muscle actin (α SMA)-expressing myofibroblasts (Fig. 3d), cells that produce CXCL13¹⁶. Oxaliplatin-induced IgA $^{+}$ B cells from spleen and MC tumors expressed activation-induced cytokine deaminase (AID) (Fig. 9f,g), suggesting recent occurrence of class-switch recombination (CSR).

The IgA CSR is induced mainly by TGF β in combination with CD40L, IL-21, IL-10 or IL-6^{17,18}. Accordingly, oxaliplatin treatment increased the proportion of tumoral B cells undergoing TGF β signaling, including phosphorylated SMAD2/3 in B cells and higher *Tgfb1* mRNA in tumors (Fig. 3e; Fig. 9h-j). IL-21 ensures TGF β -induced IgA CSR, via STAT3 signaling¹⁸. Indeed, oxaliplatin treatment increased IL-21 expression and enhanced STAT3 phosphorylation in tumoral B cells (Fig. 9k,l). Oxaliplatin also increased *Il10* mRNA expression in tumors, the number of tumor-infiltrating IL-10 producing B cells and the amount of IL-10 per B cell (Fig. 3f,g; Fig. 9m), mainly in IgA $^{+}$ CD19 $^{+}$ plasmocytes (Fig. 3h-j), 50% of which contained both IL-10 mRNA and protein (Fig. 9n). Oxaliplatin also induced Fas ligand (Fas-L) and PD ligand 1 (PD-L1), and about 50% of the IgA $^{+}$ B cells

expressed both molecules (Fig. 3k,l, Fig. 8f-i). Similarly, about 40% of oxaliplatin-induced IgA⁺ cells expressed both PD-L1 and IL-10 (Fig. 8j). Some PD-L1⁺ B cells (nearly 90% of which were IgA⁺) showed SMAD2/3 phosphorylation (Fig. 9j). Notably, LTα/β-producing B cells did not express IL-10 and their abundance was barely increased by oxaliplatin (Fig. 9o,p). CD19⁺ cells from either untreated or oxaliplatin-treated tumors did not express CD5, a marker of B regulatory (Breg) cells¹⁹ (Fig. 9q). Oxaliplatin treatment induced other immunoregulatory molecules, including Nos2, Arg1, IL-12p35 and IL-12p40, but no differences were observed between tumor-bearing WT and *Jh*^{-/-} mice, although the latter expressed higher levels of anti-tumorigenic²⁰ IL-12 (Fig. 10a-d). B cell deficiency or depletion had no significant effect on tumoral NK cells, myeloid CD11b⁺Gr1⁺ cells, macrophages or Treg (Fig. 10e-i). Thus, unlike mouse squamous cell carcinoma, where B cells modulate tumor growth and therapeutic responsiveness through effects on macrophages²¹, B cells in murine PC impede immunogenic chemotherapy by suppressing CTL activation.

EXAMPLE 5: IgA⁺ plasma cells in human prostate cancer

Human PC samples (n=110) were analyzed for CD8⁺ T cells and CD20⁺ B cells (Fig. 11a,b). Comparison of CD8 and CD20 immune reactive areas (IRA) in matched normal versus tumor tissue specimens from 87 early stage PC (E-PC) patients indicated higher CD8⁺ and CD20⁺ cell counts in tumor tissue (Fig. 11c,d). Moreover, patients with therapy-resistant PC (TR-PC) or metastatic PC (M-PC) exhibited reduced tumoral CD8⁺ cell density relative to E-PC patients (Fig. 11e). By contrast, E-PC contained fewer B cells than TR-PC and M-PC, where B cells were most abundant (Fig. 11f). E-PC specimens displayed higher CD8/CD20 ratio and lower CD20/CD8 ratio than TR-PC and M-PC (Fig. 11g,h). Immunofluorescence (IF) and immunohistochemical (IHC) analyses of human PC specimens revealed IgA⁺ cells in a scattered formation, especially in the high risk group, frequently next to αSMA⁺ myofibroblasts (Fig. 3m; Fig. 11i-k, and o), as found in mice. CD20⁺ B cells were found both scattered and clustered in lymphoid follicle-like¹⁶ areas (Fig. 11b,l). IL-10-producing IgA⁺ CD138⁺ cells were also detected in human PC and some IgA⁺ cells were adjacent to CD8⁺ T cells and expressed little CD20 (Fig. 11j-l). Approximately 25% of the IgA⁺ cells in fresh prostatectomy specimens expressed IL-10 and they were enriched in the malignant portion of the prostate (Fig. 11m,n). IgA⁺CD138⁺ plasmocytes exhibited higher density in TR-PC and M-PC than in E-PC (Fig. 11o,p) and patients with higher IgA⁺CD138⁺ cell counts showed lower CD8/CD20 ratio (Fig. 11q). *In silico* analysis of human IgA (*IGHA1*) mRNA in PC Oncomine datasets²² revealed elevated *IGHA1*

mRNA in malignant versus healthy prostates in 11 of 15 datasets. Of these, 5 showed a significant increase ($p < 0.05$) and 3 showed a > 2 -fold change. Results of one analysis²³ are presented (Fig. 11r) and are consistent with earlier findings regarding lymphocyte infiltration and activation of the IKK α -Bmi1 module by B cells in mouse PC^{7,8,24}, suggesting that tumor infiltrating lymphocytes also control malignant progression and response to therapy in human PC.

EXAMPLE 6: Immunosuppressive plasmacytes depend on TGF β signaling, PD-L1 and IL-10

We examined mechanisms responsible for generation of tumoral IgA⁺ plasmacytes and their immunosuppressive function. Consistent with prior knowledge regarding the IgA CSR^{17,25} and presence of activated SMAD2/3 in PD-L1-expressing B cells, TGF β receptor II (TGF β R2)²⁶ ablation in B cells (*Tgfb β r2^{ΔB}*) enhanced the response of MC tumors to oxaliplatin (Fig. 4a), decreased the number of tumor-infiltrating, but not splenic, B cells (Fig. 4b and Fig. 12a) and inhibited oxaliplatin-induced IgA⁺ plasmacyte generation without affecting IgG1⁺ or IgG2a⁺ cells (Fig. 4c; Fig. 12b-d). IgA ablation also potentiated the response to oxaliplatin without reducing tumoral B cell number (Fig. 4a,b). Both TGF β R2 and IgA ablations increased serum IgG independently of oxaliplatin, prevented appearance of tumoral PD-L1⁺ or IgA⁺IL-10⁺ B cells after oxaliplatin treatment, but had almost no effect on IL-10 production by B220^{hi}IgA⁺ B cells (Fig. 12e-g). TGF β R2 ablation in B cells or IgA deficiency also increased tumoral CTL density, tumoral IFN γ -production and surface CD107a expression by CD8⁺ T cells isolated from oxaliplatin-treated mice after *ex vivo* stimulation with MC lysates or PMA/ionomycin (Fig. 4e,f). These results are consistent with clinical observations, describing systemic and organ-specific autoimmune diseases as major manifestations of IgA deficiency²⁷, which suggest that IgA⁺ cells suppress activation of auto-reactive T cells.

Suppressor B cells may attenuate T cell activation via PD-L1²⁵. Treatment of mice bearing MC tumors with a combination of oxaliplatin and anti-PD-L1, but not anti-PD-L1 alone, inhibited tumor growth, increased GrzB expression by effector T cells, downregulated PD-L1 expression on IgA⁺ B cells, and reduced serum IgA, but not IgG (Fig. 12h-m). Furthermore, reconstitution of MC tumor-bearing *Jh^{-/-}* hosts with B cells deficient in either PD-L1 or IL-10 failed to inhibit oxaliplatin-induced tumor regression (Fig. 4g,h; Fig. 12n,o). PD-L1 ablation did not affect IL-10 expression and IL-10 ablation had no effect on PD-L1 (Fig. 15j,k), indicating that both molecules are needed for plasmacyte-mediated immunosuppression.

All of the above experiments used LD oxaliplatin because of its well-described immunogenic properties^{4,5}. The related compound cisplatin, however, is devoid of immunogenic activity^{4,5,28}. Although both oxaliplatin and cisplatin induced apoptotic cell death of MC cells, oxaliplatin, was more effective in stimulating autophagy (Fig. 13a,b). Importantly, only LD oxaliplatin led to regression of MC tumors raised in *Jh*^{-/-} mice, whereas LD cisplatin was ineffective (Fig. 13c). Correspondingly, only oxaliplatin increased the abundance of tumor infiltrating CD8⁺ and CD4⁺ cells in *Jh*^{-/-} mice (Fig. 13d,e). LD, but not high dose, oxaliplatin did not increase intestinal permeability and had no effect on IgA production and other immune parameters in tumor-free WT or *Tgfb β 2*^{Ab} mice (Fig. 13f-k).

EXAMPLE 7: Adoptively transferred T cells eliminate tumors in the absence of B cells

We examined whether immunogenic chemotherapy also potentiates the effectiveness of adoptively transferred T cells (ATCT). We grew immunogenic TRAMP-C2 PC cells²⁹ in B cell-containing *Ter β* ^{-/-} mice and combined oxaliplatin treatment with ATCT (Fig. 14a). Higher tumor volumes in *Ter β* ^{-/-} relative to WT mice confirmed TRAMP-C2 immunogenicity (Fig. 14b). However, despite successful T cell take and elevated CD8⁺ cell count after oxaliplatin no tumor rejection took place (Fig. 14c-e). Different results were obtained in *Rag1*^{-/-} mice, which lack T and B cells (Fig. 14f). We raised TRAMP-C2 tumors in *Rag1*^{-/-}; *OT-1* mice, which lack B cells and polyclonal T cells but harbor CD8⁺ T cells directed against chicken ovalbumin (Ova)³⁰. These mice were used instead of simple *Rag1*^{-/-} mice to avoid lymphopenia-induced proliferation of CD8⁺ cells, which can non-specifically increase T cell effector function³¹. Adoptively transferred CD8⁺ T cells expanded and expressed GrzB in *Rag1*^{-/-}; *OT-1* hosts, especially after oxaliplatin treatment (Fig. 14g,h). Consequently, tumor growth was inhibited in mice receiving ATCT together with oxaliplatin (Fig. 14i,j).

More dramatic results were obtained in *TRAMP*; *Rag1*^{-/-} mice transplanted with CFSE-labeled splenocytes from either naïve WT (B and T cell transfer) or *Jh*^{-/-} (T cell transfer) mice at 16 weeks of age (Fig. 5a). Six days later, CD8⁺ cell proliferation was confirmed in BM, spleens and prostates of transplanted mice, indicating successful T cell take, although lower T cell proliferation was seen when T and B cells were co-transferred (Fig. 14k,l). Thirty days after lymphocyte transfer, prostate tumors were analyzed. Oxaliplatin led to tumor regression in mice receiving T and B cells, but in mice receiving only T cells it induced complete tumor regression (Fig. 5b,c). Transplantation with both T and B cells combined with oxaliplatin treatment led to appearance of CD19⁺ B cells in spleen and

prostate and restored serum IgA and IgG (Fig. 5d-f). IgA and IgG directed against SV40 T antigen, the *TRAMP* oncogene, were detected after B and T cell transfer, indicating a tumor-specific humoral response (Fig. 5g,h). Transferred B cells showed higher expression of TIM-1 (Fig. 14m,n), a molecule involved in regulation of IL-10 expression and tolerance induction³². Co-transplantation of B and T cells also led to appearance of CD4⁺ and CD8⁺ cells in lymph nodes and spleen, but T cell number was considerably lower in prostate tumors (Fig. 5i; Fig. 14o-q). However, transplantation with B cell-deficient splenocytes led to robust T cell infiltration into prostate tumors (Fig. 5i; Fig. 14q). To further confirm that IgA⁺ B cells attenuate the response to immunogenic chemotherapy by inhibiting T cell activation, we raised MC tumors in *Rag1*^{-/-} mice and transplanted the mice with T cells from WT mice that were immunized with MC cell lysate, with or without naïve splenic B cells from WT or *Tgfb β 2^{ΔB}* mice. In this case, oxaliplatin induced tumor regression and CTL activation only in mice receiving T cells, or T cells + TGFβ2-deficient B cells, which produced just a low amount of IgA (Fig. 5j-l; Fig. 14r-t). Hence, only B cells that have undergone TGFβR signaling and IgA CSR suppress CTL activation.

EXAMPLE 8: Anti-PD-L1 treatment induce hepatocellular carcinoma tumor regression only when combined with oxaliplatin.

Liver cancer rates are rapidly growing while all other cancers are declining (Ryerson et al. (2016), Annual Report to the Nation on the Status of Cancer, 1975-2012, Cancer. doi: 10.1002/cncr.29936). Figure 16 shows that immunosuppressive B cells are indeed also present in liver cancer, and the combination therapy of oxaliplatin with checkpoint inhibitor (aPDL1) decreased the number of liver tumors.

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CLAIMS

We claim:

- 5 1. A method for treating epithelial cancer in a mammalian subject in need thereof, wherein said subject comprises cancer tissue that contains epithelial cancer cells and immunosuppressive B cells, and wherein said method comprises administering to said subject a therapeutically effective amount of
- a) one or more first composition that causes immunogenic cell death, and
 - b) one or more second composition that reduces one or both of the number and function of
- 10 said immunosuppressive B cells in said cancer.
2. The method of Claim 1, wherein said first composition that causes immunogenic cell death is selected from the group consisting of oxaliplatin, cyclophosphamide, and mitoxantrone, anthracyclines, and bortezomib.
- 15 3. The method of Claim 2, wherein said first composition that causes immunogenic cell death comprises oxaliplatin.
4. The method of Claim 1, wherein said second composition that reduces one or both of the
- 20 number and function of said immunosuppressive B cells in said cancer comprises an antibody that specifically binds to said immunosuppressive B cells.
5. The method of Claim 4, wherein said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer comprises anti-CD19 antibody
- 25 and anti-CD20 antibody.
6. The method of Claim 4, wherein said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer comprises anti-PDL1 antibody.

7. The method of Claim 1, wherein said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer comprises anti-CD19-CAR T cells.

8. The method of Claim 1, wherein said immunosuppressive B cells comprise TGF beta receptors, and wherein said second composition that reduces one or both of the number and function of said immunosuppressive B cells in said cancer is a compound that inhibits binding of said TGF beta receptor to a TGF beta receptor ligand.

9. The method of Claim 8, wherein said second composition that inhibits binding of said TGF beta receptor to a TGF beta receptor comprises ALK5 inhibitor.

10. The method of Claim 1, wherein said epithelial cancer is selected from the group consisting of prostate cancer, liver cancer, bladder cancer, lung cancer, and cutaneous melanoma.

11. The method of Claim 10, wherein said prostate cancer is selected from the group consisting of castrate-resistant prostate cancer (CRPC), recurrent prostate cancer, and metastatic prostate cancer.

12. The method of Claim 1, wherein said epithelial cancer is selected from the group consisting of primary cancer, recurrent cancer, and metastatic cancer.

13. The method of Claim 1, wherein said administering comprises administering said first composition and said second composition substantially simultaneously.

14. The method of Claim 1, wherein said administering comprises administering said first composition and said second composition sequentially in any order.

15. The method of Claim 1, wherein said administering comprises administering said first composition and said second composition substantially simultaneously and sequentially in any order.

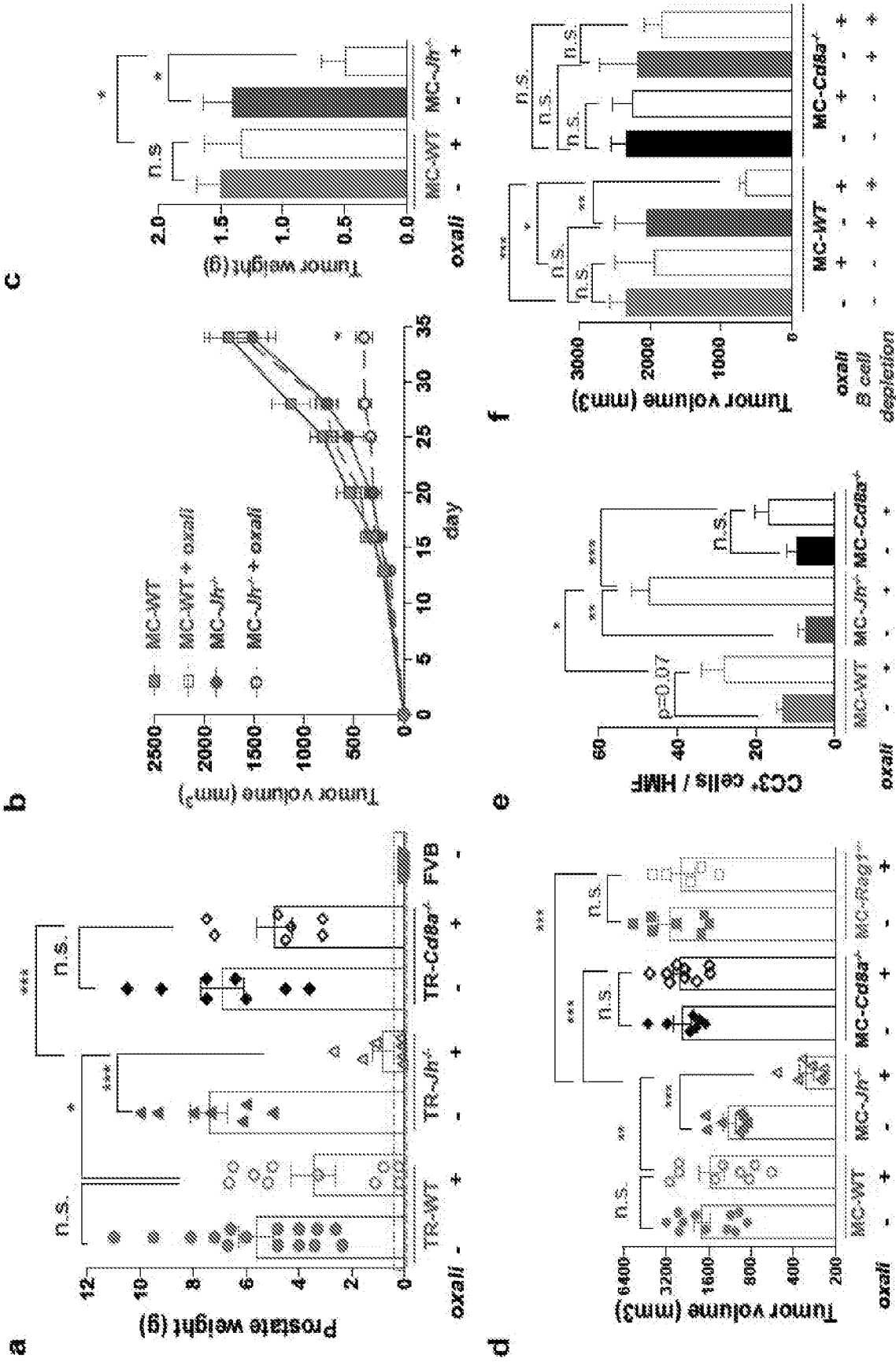


FIGURE I

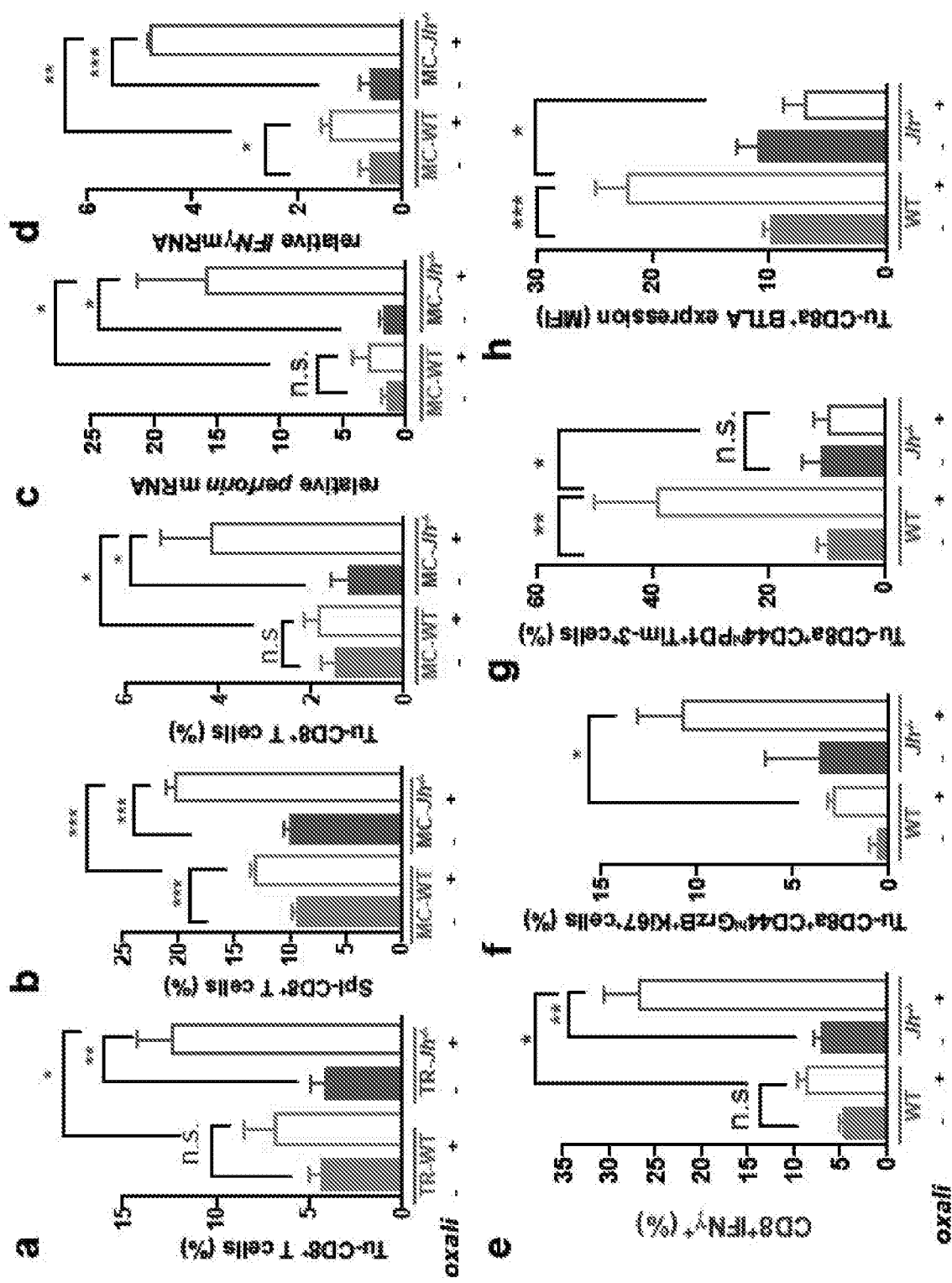


FIGURE 2

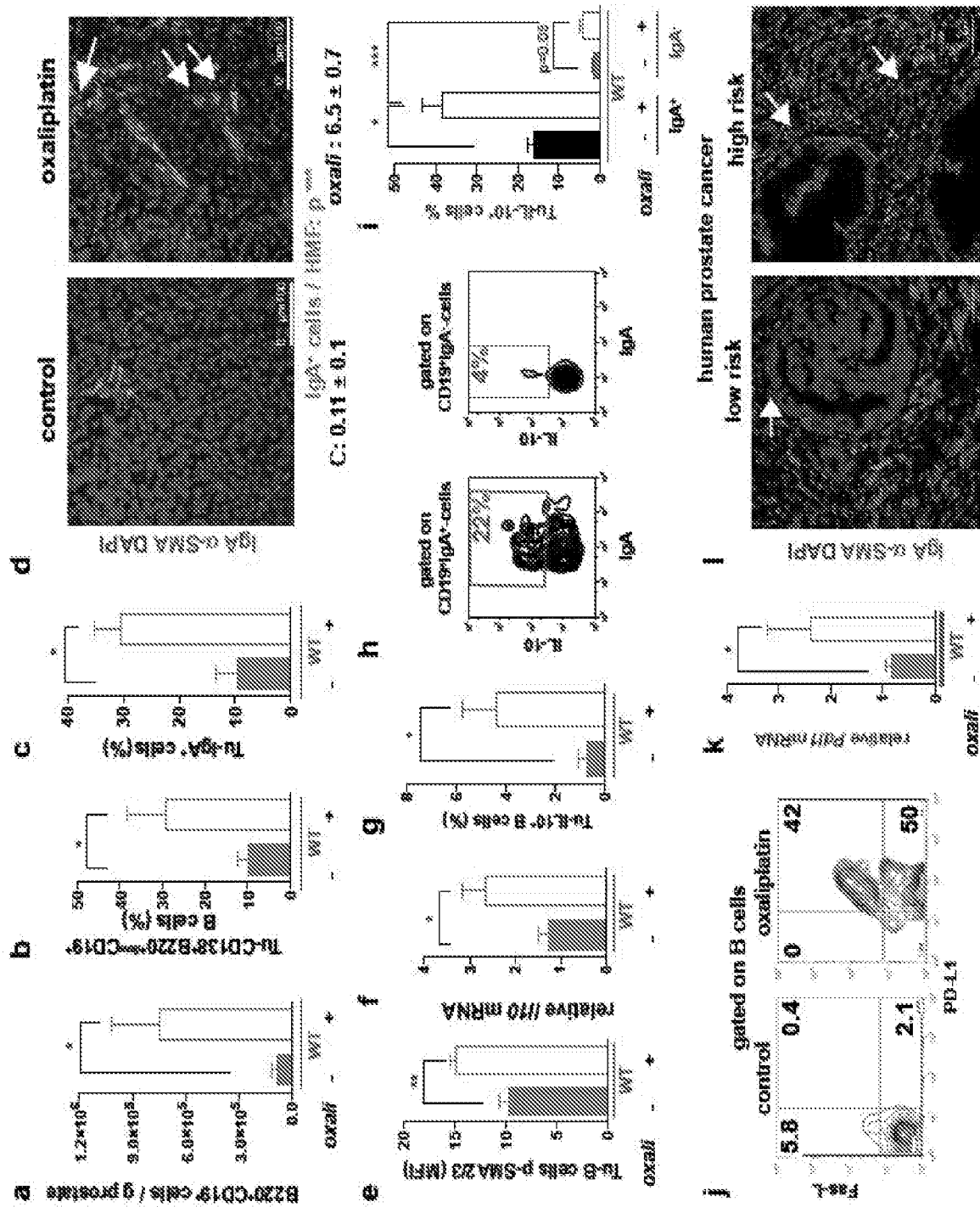


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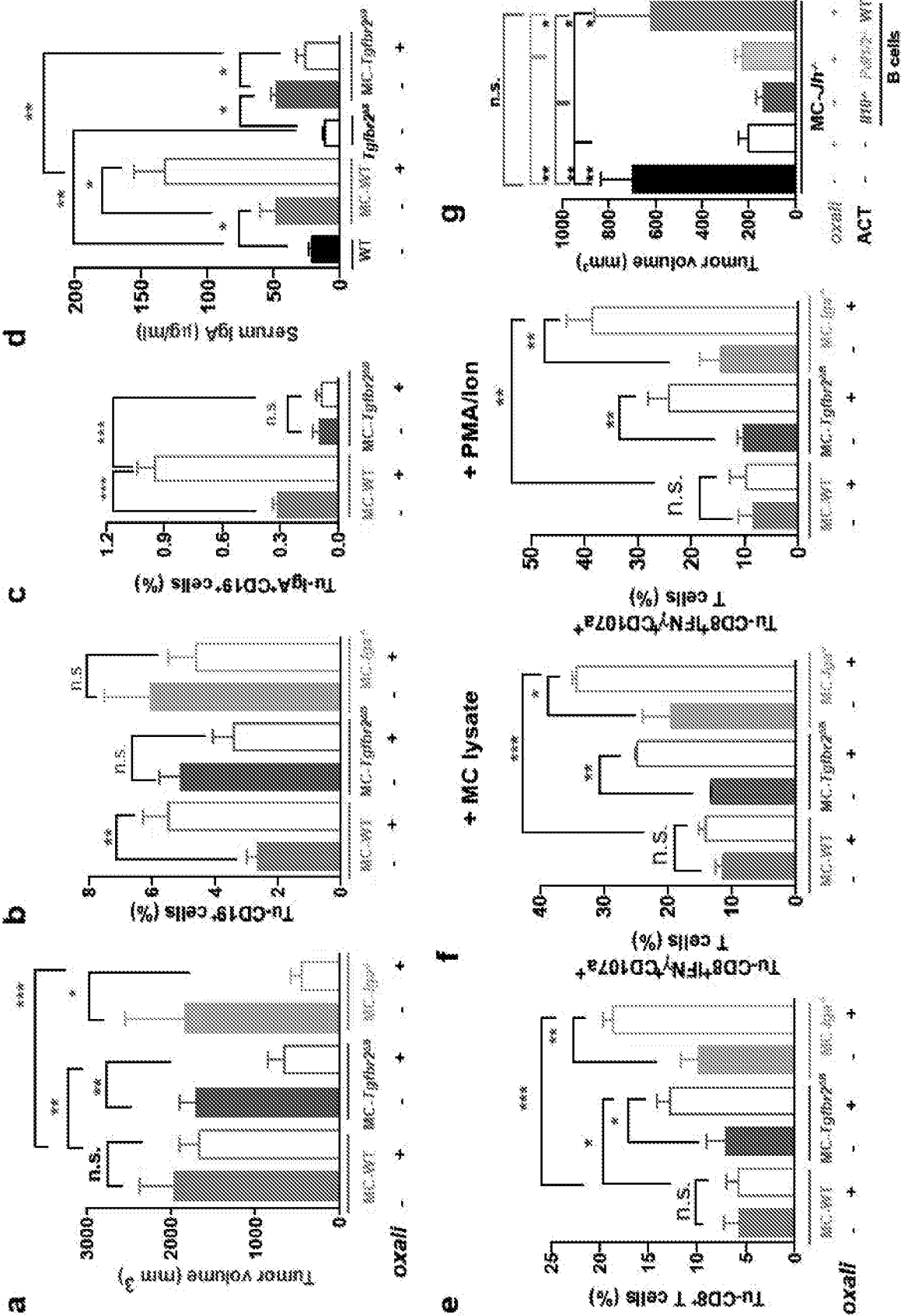


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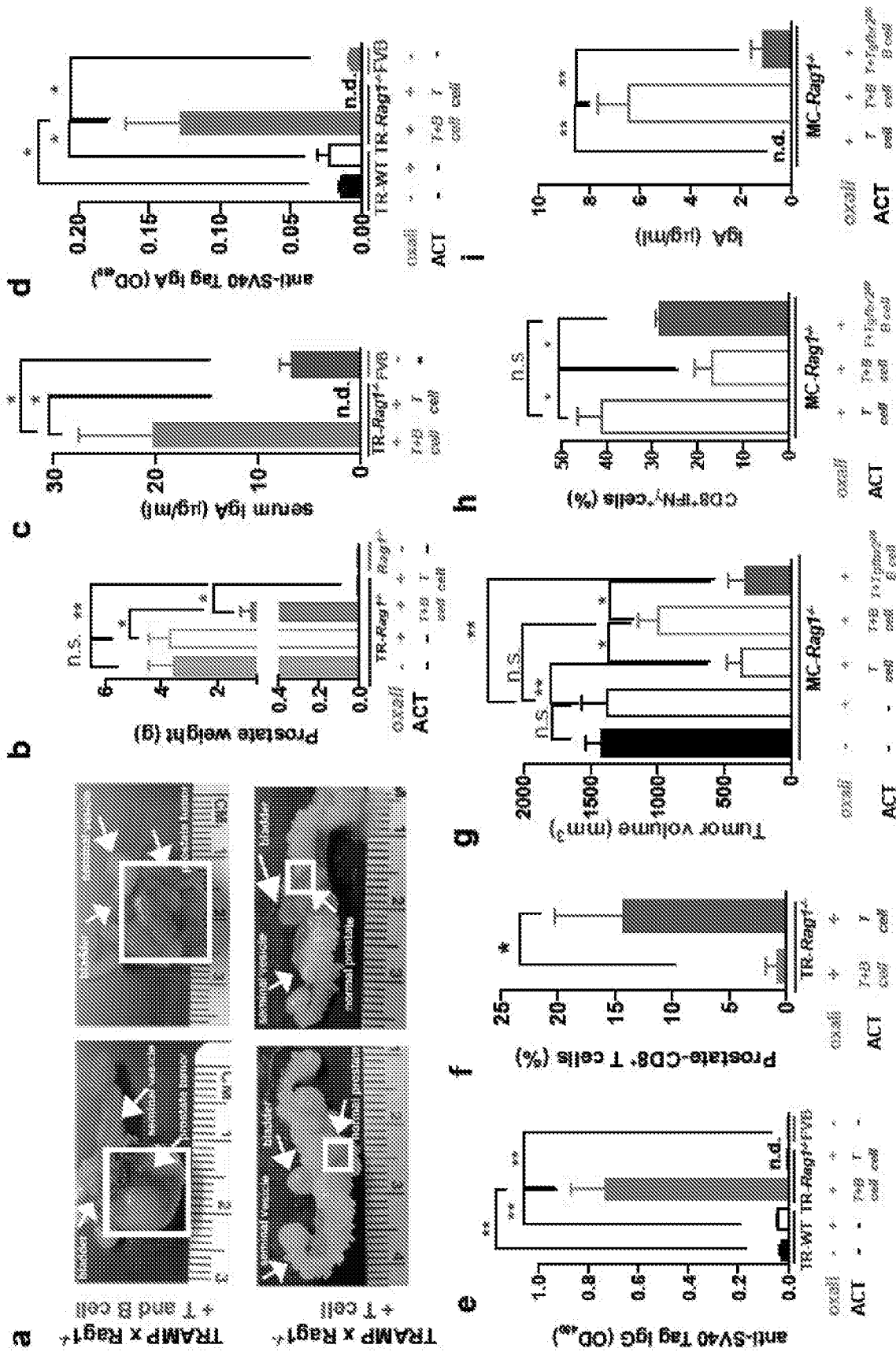
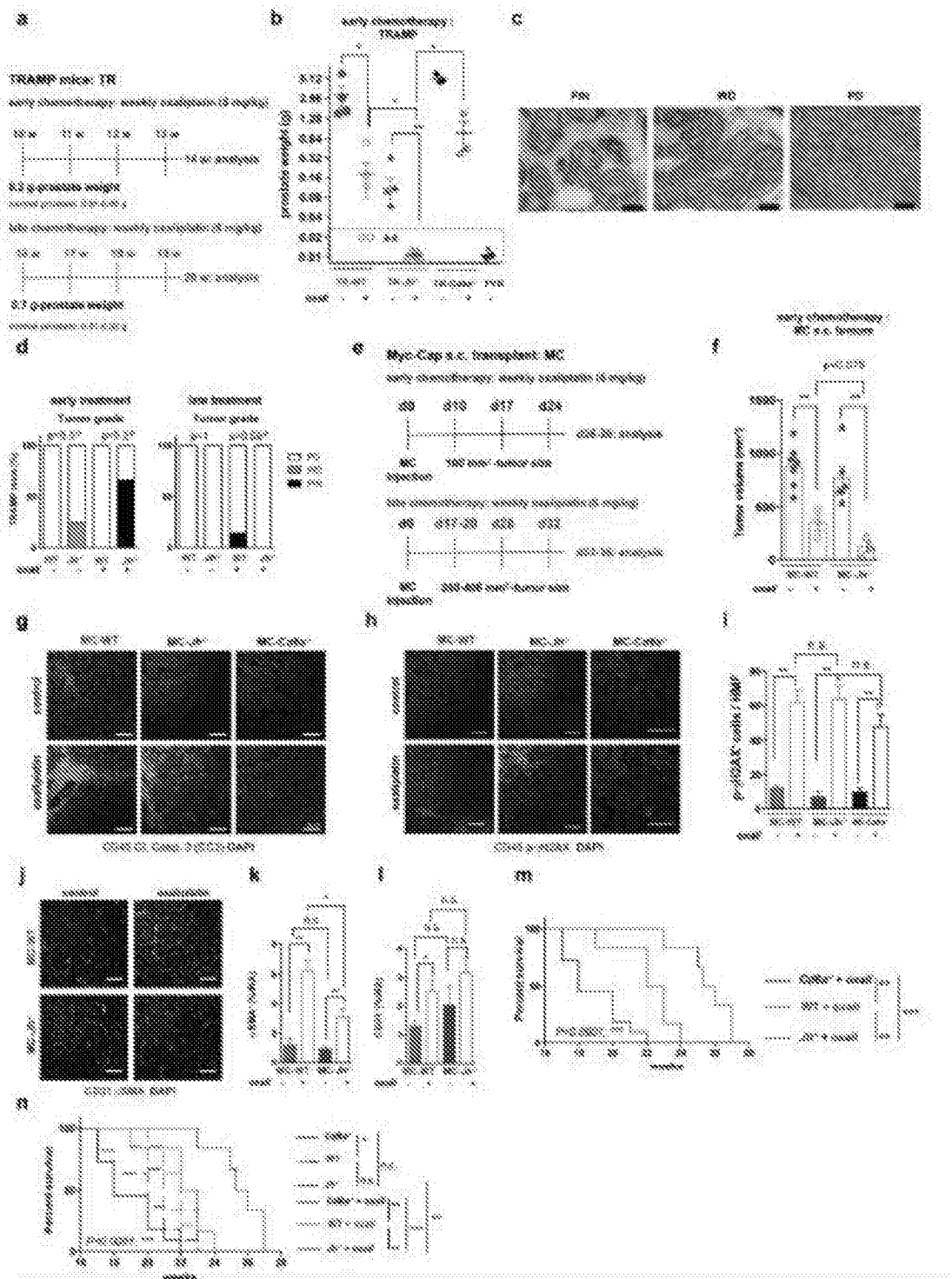


FIGURE 5



FIGURES 6

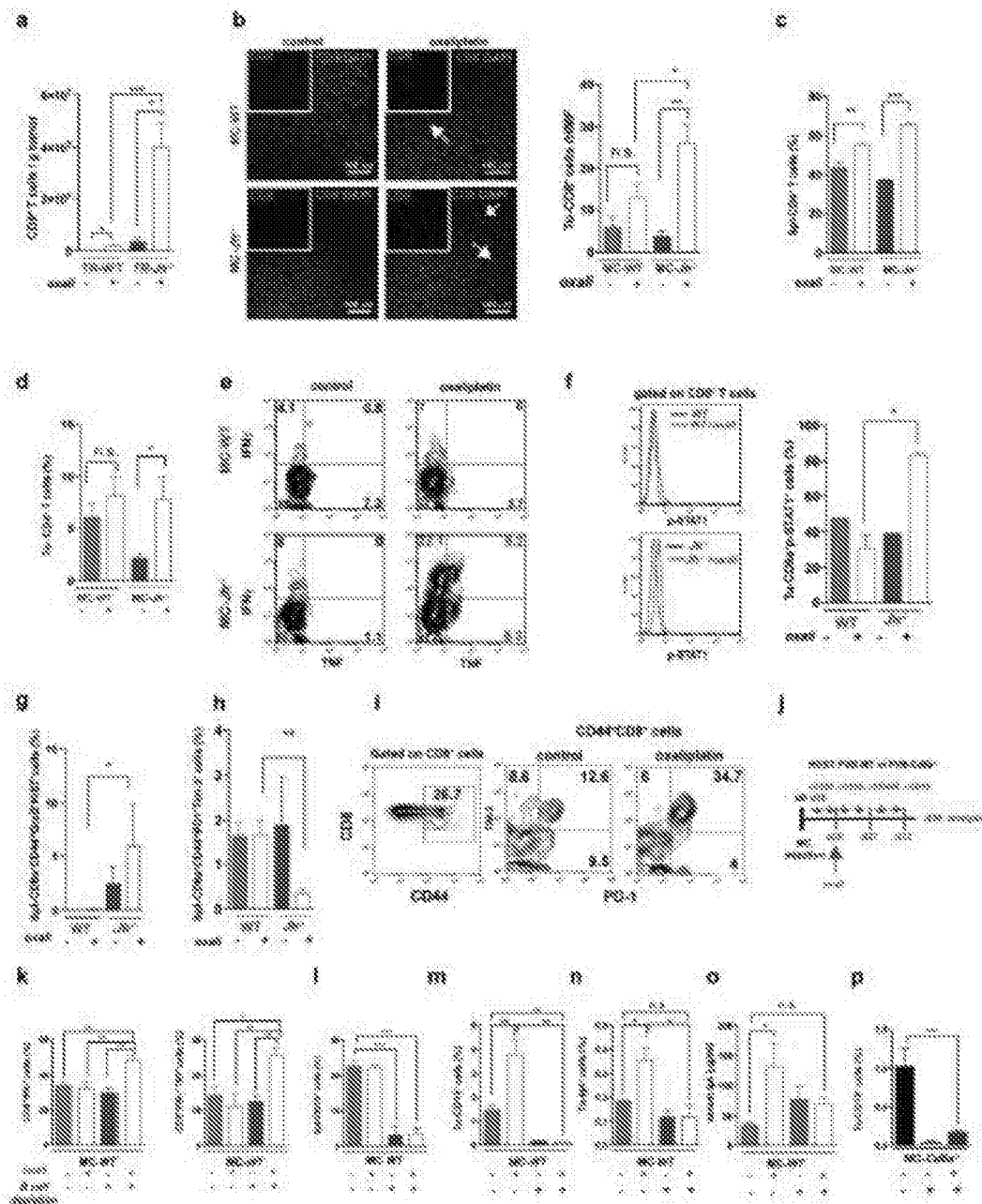


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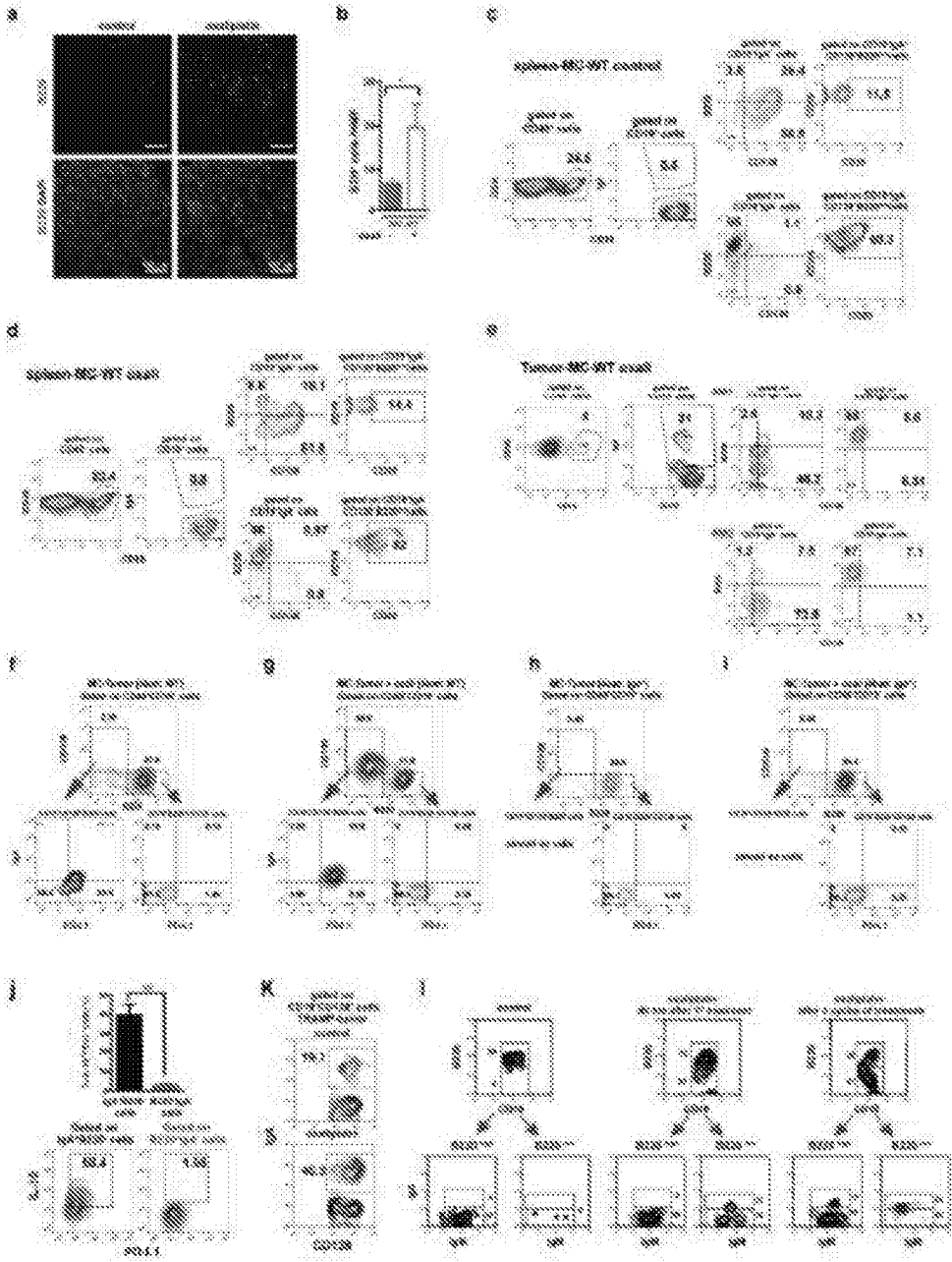


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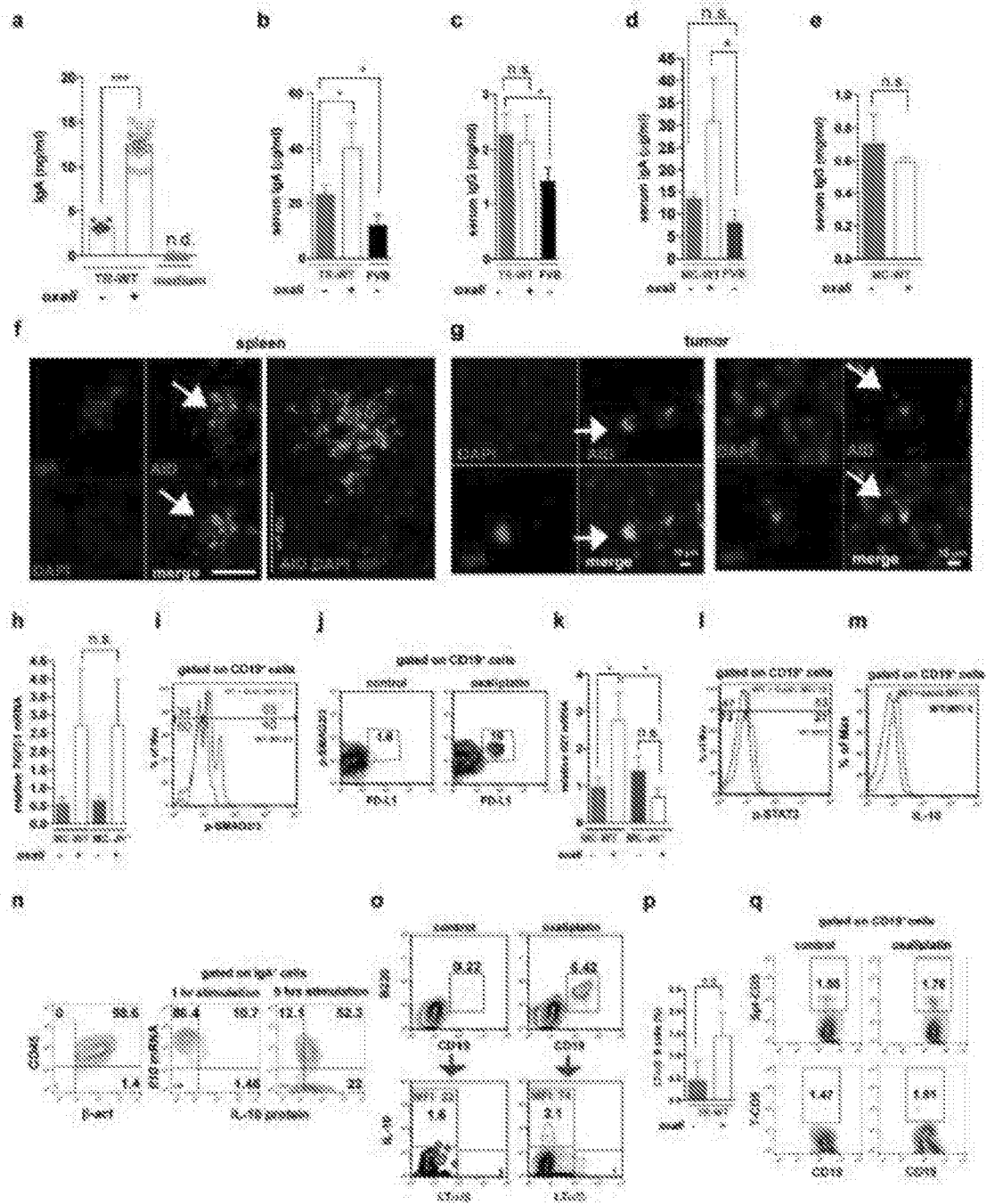


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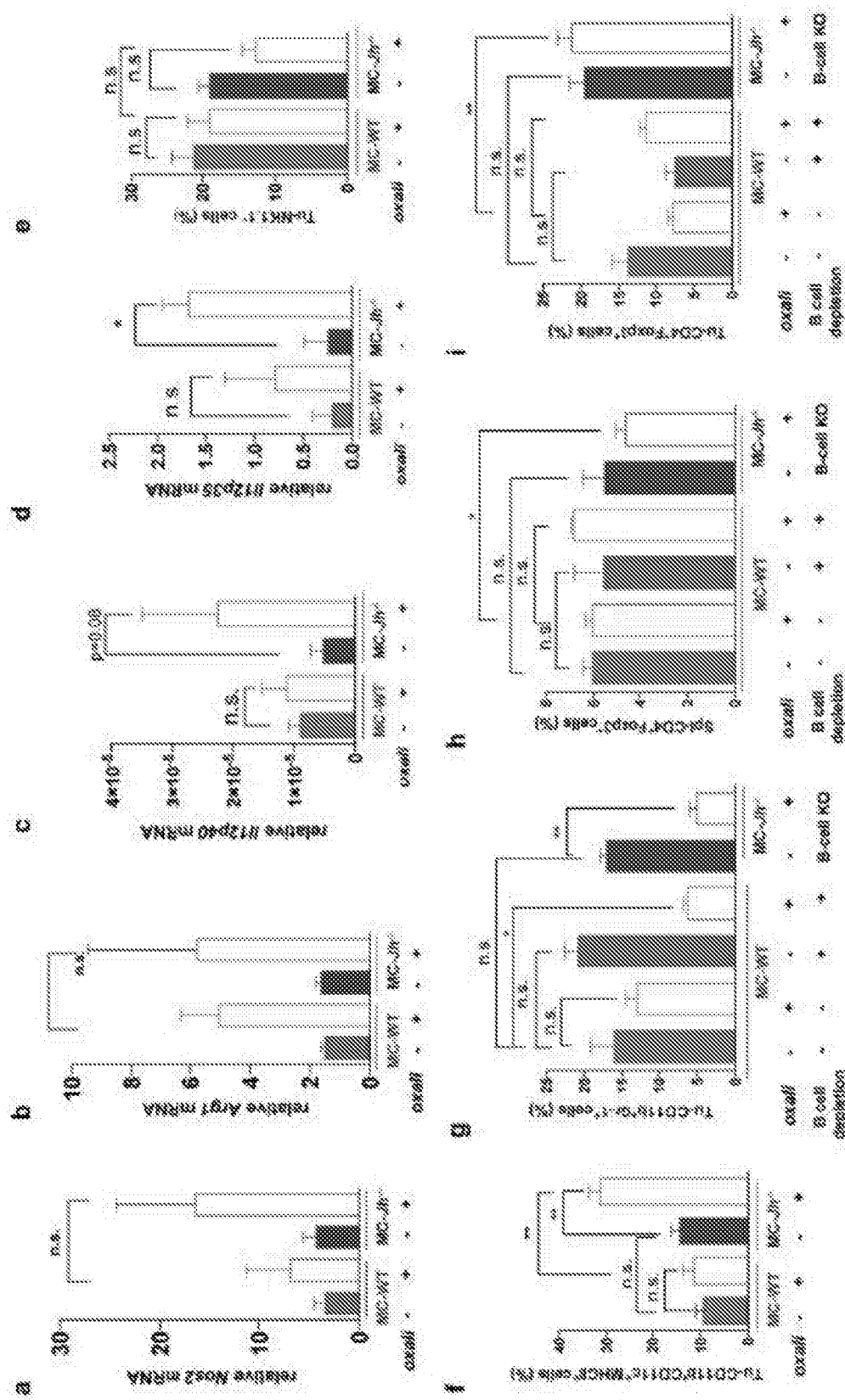
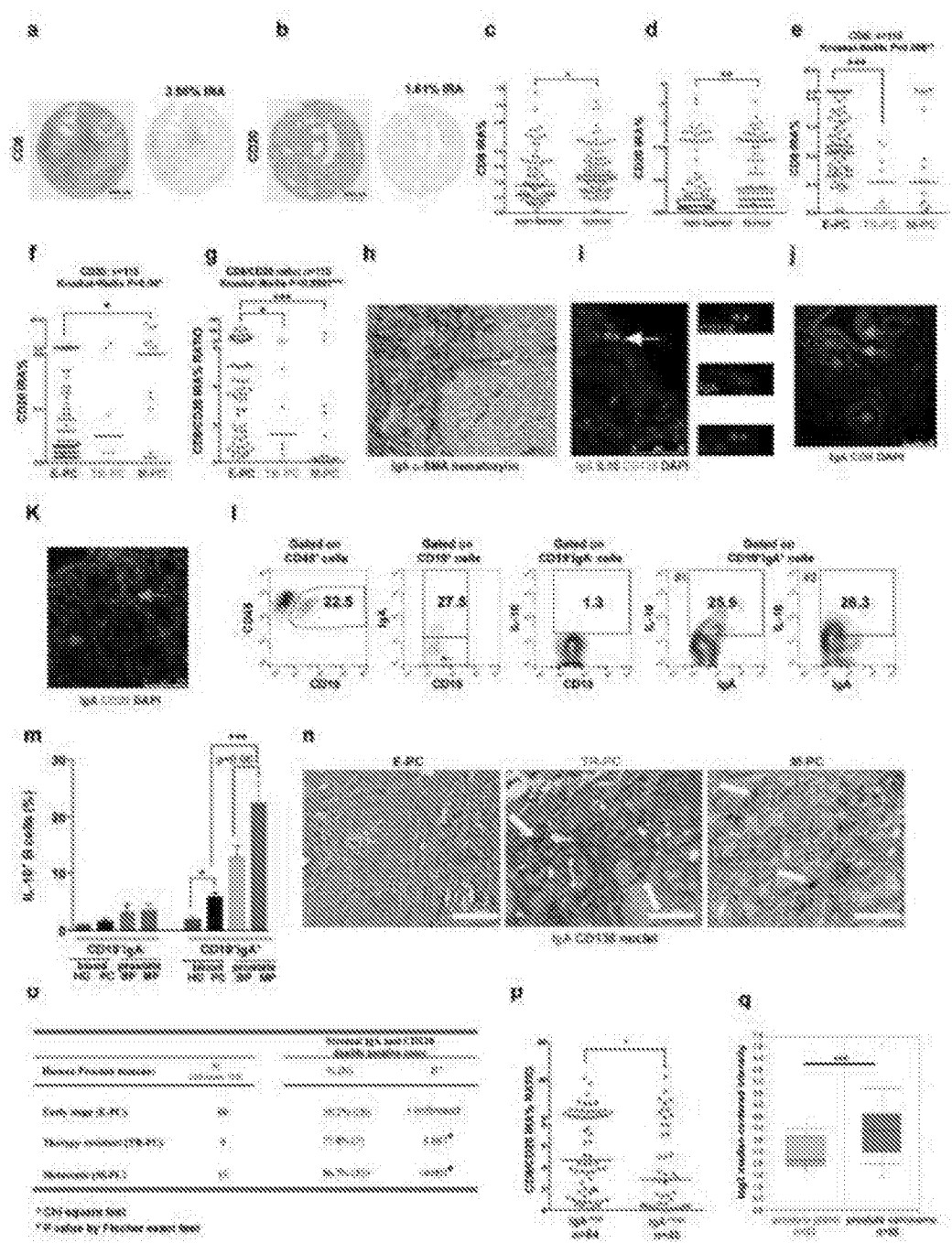


FIGURE 10



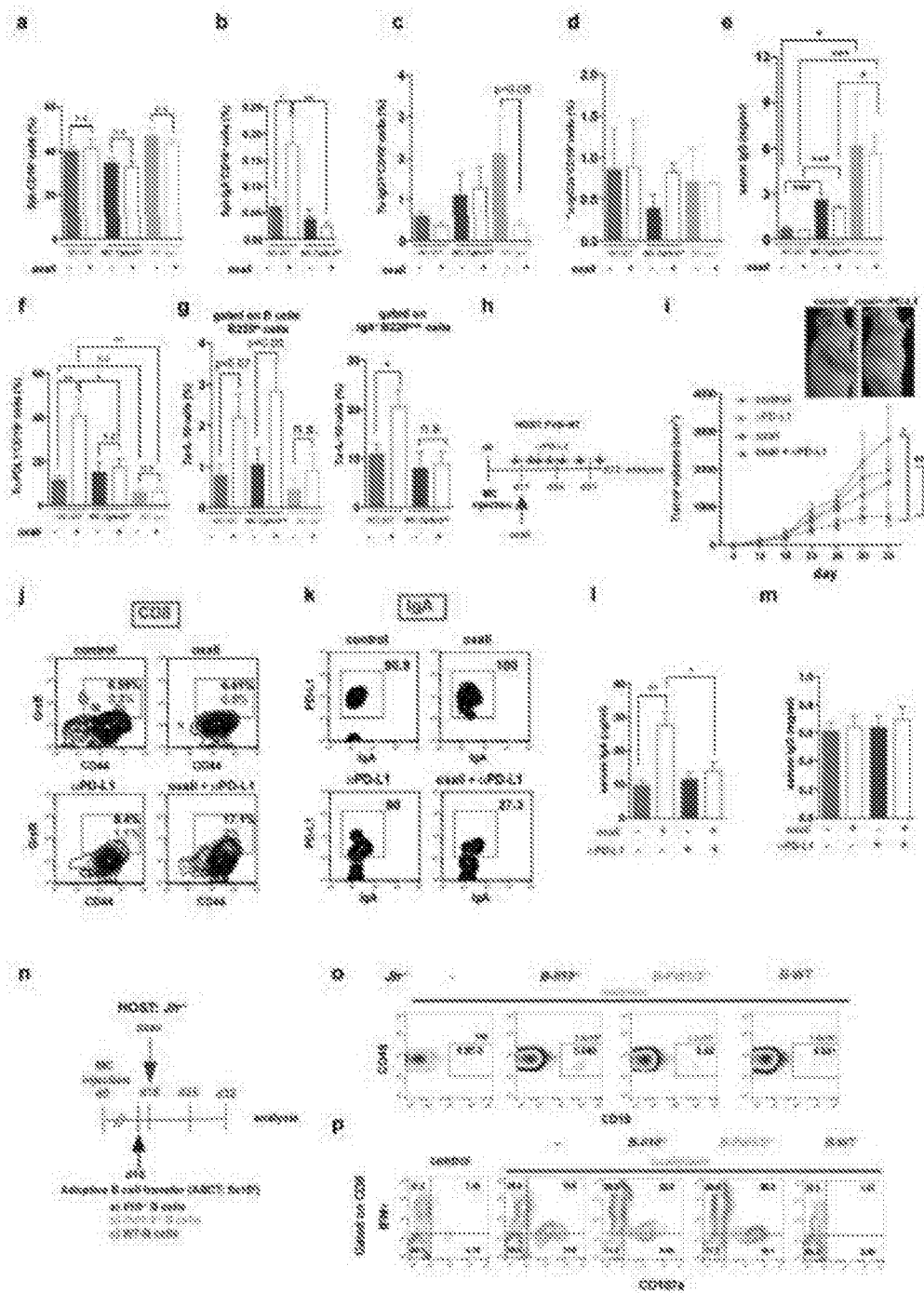


FIGURE 12

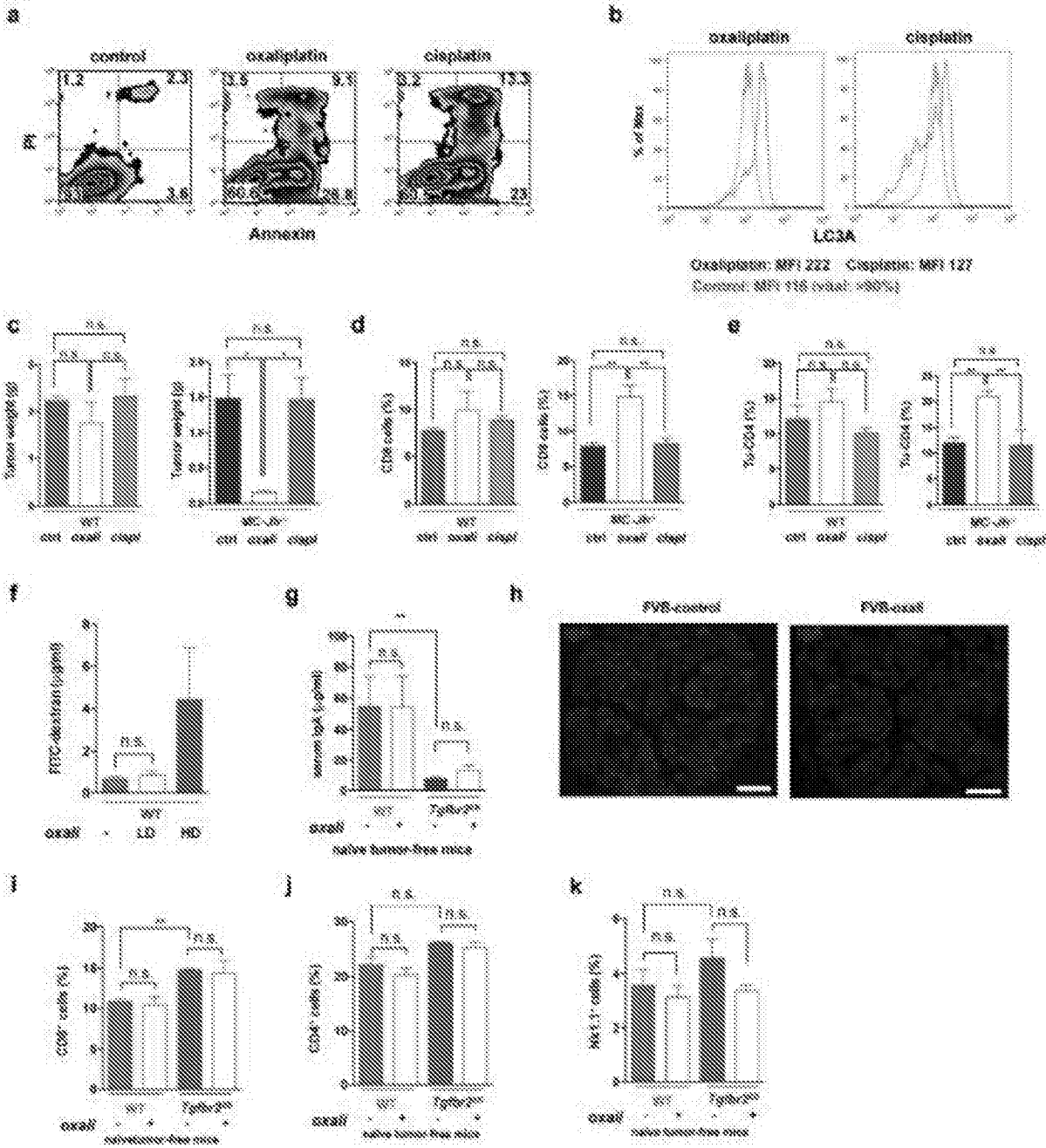


FIGURE 13

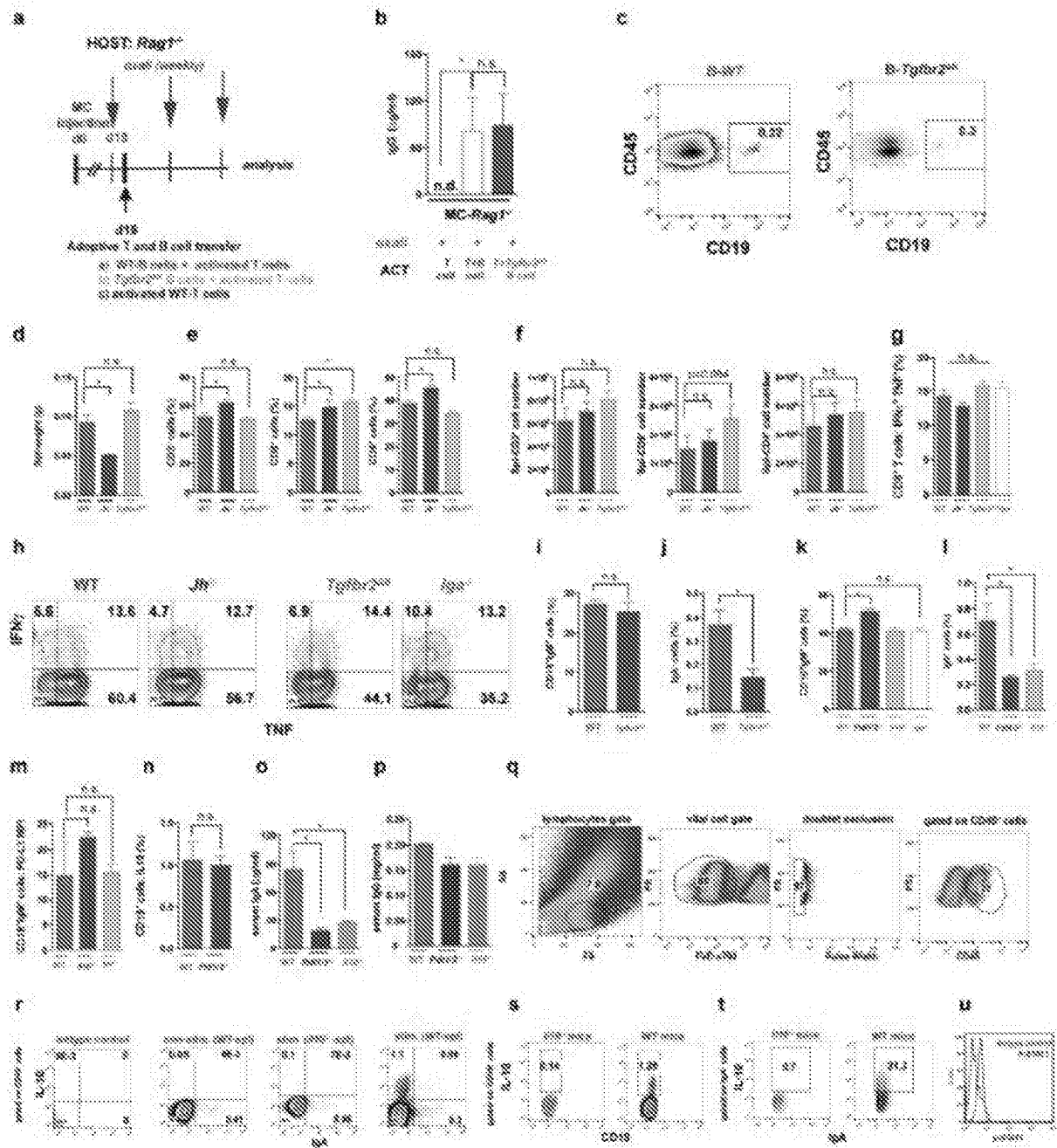


FIGURE 15

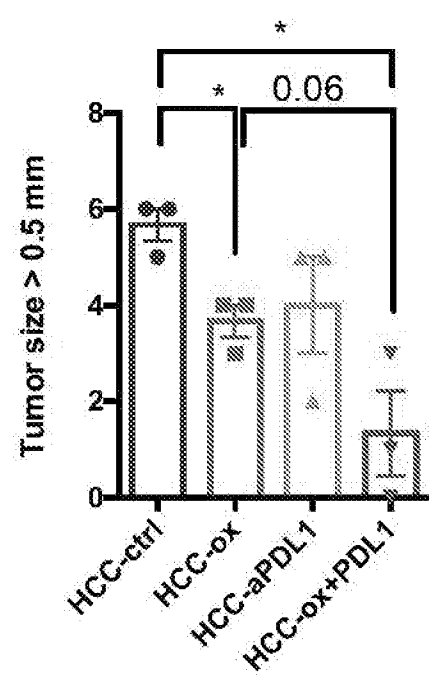


Figure 16

Amino acid sequence of programmed cell death 1 ligand 1 isoform a precursor (PD-L1) Homo sapiens) SEQ ID NO:01-04

A. NCBI Reference Sequence: NP_054862.1 (SEQ ID NO:01)

```

1  mrifavfifm tywhllnaft vtvpkdlyvv eygsnmtiec kfpvekqldl aalivyweme
61  dkniiqfvhg eedlkvqhss yrqrarllkd qlslgnaalq itdvklqdag vyrcmisygg
121 adykritvkv napynkinqr ilvvdptvse heltcqaegy pkaeviwts dhqvlsgktt
181 ttnskreekl fnvtstlrin tttneifyct frildpeen taelvipelp lahppnerth
241 lvilgaillo lgvaltfifr lrkgrmmdvk kogiqdtnsk kgsdthleet

```

B. Entrez Gene: 29126, NCBI Reference Sequence: NC_000009.12 (SEQ ID NO:02)

```

MRIFAVFIFMTYWHLNNAFTVTVPKDLVVEYGSNMTIECKFPV
EKQLDLAALIVWEMEDKNIIQFVHG EEDLKVQHSSYRQRARLLKDQLSLGNAALQIT
DVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPTVSEHELTCAEGY
PKAEVIWTS SDHQVLSGKTTT TNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEE
NHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQD
TNSKKQSDTHLEET

```

C. Entrez Gene: 29126, NCBI Reference Sequence: NC_000009.12 (SEQ ID NO:03)

```

MRIFAVFIFMTYWHLNAPYNKINQRILVVDPTVSEHELTCAE
GYPKAEVIWTS SDHQVLSGKTTT TNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDP
EENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIERLRKGRMMDVKKCGI
QDTNSKKQSDTHLEET

```

D. Entrez Gene: 29126, NCBI Reference Sequence: NC_000009.12 (SEQ ID NO:04)

```

MRIFAVFIFMTYWHLNNAFTVTVPKDLVVEYGSNMTIECKFPV
EKQLDLAALIVWEMEDKNIIQFVHG EEDLKVQHSSYRQARLLKDQLSLGNAALQIT
DVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPTVSEHELTCAEGY
PKAEVIWTS SDHQVLSGD

```

Figure 17

Amino acid sequence of interleukin 10 (IL10) (Homo sapiens) NP_00563.1 ; Entrez Gene: 3586
(SEQ ID NO:05):

MHSSALLCCLVLLTGVRASPGQGTQSENSCTHFPGNLPNMLRDL

RDAFSRVKTFQMKDQLDNLLLKESLLEDFKGYLGCAALSEMIQFYLEEVMPPQAENQD

Figure 18

A. IGHA1_HUMAN, P01876, Entrez Gene: 3493, NCBI Reference Sequence: NC_000014.9, containing Ig alpha-1 chain C region, Binding site P01876[352] (SEQ ID NO:06)

ASPTSPKVFP LSLCSTQPDG NVVIACLVQG FFPQEPLSVT WSESGQGVT
 RNFPSSQDAS GDLYTTSSQL TLPATQCLAG KSVTCHVKHY TNPSQDVTVP
 CPVPSTPPTP SPSTPPTPSP SCCHPRLSLH RPALEDLLG SEANLTCTLT
 GLRDASGVTF TWTPSSGKSA VQGPPERDLC GCYSVSSVLP GCAEPWNHGK
 TFTCTAAYPE SKTPLTATLS KSGNTRPEV HLLPPPSEEL ALNELVLTLC
 LARGFSPKDV LVRWLQGSQE LPREKYLTWA SRQEPSQGT TFAVTSILRV
 AAEDWKKGDT FSCMVGHEAL PLAFTQKTID RLAGKPTHVN VSVVMAEVDG
 TCY

B. IGHA2_HUMAN, Entrez Gene: 3494, P01877, containing Ig alpha-2 chain C region P01877 [1-340], PRO_0000153567 (SEQ ID NO:07)

ASPTSPKVFP LSLDSTPQDGNVVVACLVQGF FFPQEPLSVTWSESGQNV TARNFPSSQDAS
 GDLYTTSSQL TLPATQCPDG KSVTCHVKHY TNPSQDVTVP CPVPPPPCCHPRLSLHRPA
 LEDLLGSEANLTCTLT GLRDASGATFTWTPSSGKSA VQGPPERDLC GCYSVSSVLP GCA
 QPWNHGETFTCTAAHPELKTPLTANITKSGNTRPEV HLLPPPSEELALNELVLTCLAR
 GFSPKDV LVRWLQGSQELPREKYLTWASRQEPSQGT TFAVTSILRVAAEDWKKGDT FSC
 MVGHEALPLAFTQKTID RMAGKPTHVNVSVVMAEVDGTCY

Figure 19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/22855

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 39/395, 31/282, 45/06; A61P 35/00; C07K 16/28 (2016.01) CPC - A61K 39/3955, 31/282, 45/06; C07K 16/2896 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC(8) Classifications: A61K 39/395, 31/282, 31/555, 45/06; A61P 31/00, 35/00; C07K 16/28 (2016.01) CPC Classifications: A61K 39/395, 31/3955, 31/282, 31/555, 45/06; C07K 16/2896 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; PubMed; EBSCO; cancer*, tumor*, oxaliplatin, cyclophosphamide, mitoxantrone, anthracyclin*, bortezomib, CD19, CD20, rituximab, anti*, epithelial, , 'B cell*', PDL1, immunosuppressiv*, atezolizumab, MPDL3280A, 'Regents of the University of California', 'Michael Karin', 'Shabnam Shalapour', prostat*		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEE, et al. Gemcitabine–Oxaliplatin Plus Prednisolone Is Active In Patients With Castration-Resistant Prostate Cancer For Whom Docetaxel-Based Chemotherapy Failed. British Journal of Cancer. 2014, Vol. 110, pages 2472-2478, doi: 10.1038/bjc.2014.204; Table 1; abstract; page 2473, second-third and seventh paragraphs; page 2475, second paragraph; page 2476, fourth and sixth-seventh paragraphs.	1-15
Y	WOO, et al. Tumor Infiltrating B-cells Are Increased In Prostate Cancer Tissue. Journal of Translational Medicine. 30 January 2014, Vol. 12, pages 1-9, doi: 10.1186/1479-5876-12-30; abstract; page 2, second-third paragraphs; page 8, fourth paragraph.	1-15
Y	US 2013/0115657 A1 (MEDIMMUNE, LLC.) 9 May 2013; paragraphs [0014], [0025], [0031], [0053], [0327], [0329], [0372], [0479], [0614], [0616].	4-5
Y	AMMIRANTE, et al. Tissue Injury And Hypoxia Promote Malignant Progression Of Prostate Cancer By Inducing CXCL13 Expression In Tumor Myofibroblasts. PNAS. 14 October 2014, Vol. 111, pages 14776-14781, doi: 10.1073/pnas.1416498111; Significance; page 14777, first paragraph; page 14778, first paragraph; page 14781, first paragraph.	8-9
Y	ROCHE. Investor Science Conference Call From ESMO 2014. Madrid, 29 September 2014, slides 1-71; slides 55, 58, 60.	4, 6
Y	US 2014/0271635 A1 (BROGDON, et al.) 18 September 2014; paragraph [0319].	7
Y	NOWAK, et al. Gemcitabine Exerts A Selective Effect On The Humoral Immune Response: Implications For Combination Chemo-immunotherapy. Cancer Research. 15 April 2002, Vol. 62, pages 2353-2358; abstract; page 2353, fourth paragraph.	1-15
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 27 June 2016 (27.06.2016)		Date of mailing of the international search report 25 JUL 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774