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Overcoming genetically-based resistance mechanisms to PD-1 blockade

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Background

We studied loss of function (LoF) mutations within the interferon (IFN) pathway (*JAK1* or *JAK2)* and in the antigen presentation pathway (beta-2-microglobulin-*B2M*) found in biopsies from patients [1,2] who are resistance to anti-PD-1 therapy, and tested strategies to overcome the resistance. Using CRISPR/Cas9 genome editing we generated *JAK1, JAK2* and *B2M* knockout (KO) sublines of the murine MC38 colon carcinoma, a model of high mutational load cancer that responds well to anti-PD-1, as well as of human MART-1+ melanoma cell lines, tested using *in-vitro* T cell co-culture systems. We analyzed signaling changes in human cell lines (parental and KOs) exposed to IFN-gamma using RNAseq. In addition, we performed *in-vivo* antitumor activity in the MC38 model variants using mass cytometry (CyTOF) to characterize the tumor microenvironment. Finally, we tested strategies to overcome resistance mechanisms with SD-101 (TLR-9 agonist) and bempegaldesleukin (NKTR-214, a CD-122 preferential IL-2 pathway agonist). with the extent of CD4, CD8 and NK1.1 depletion.

Results

JAK1/2 LoF mutations result in insensitivity to IFN induced antitumor effects, but does not impair T cell recognition and cytotoxicity, while *B2M* LoF results in lack of antigen presentation to T cells and loss of antitumor activity.

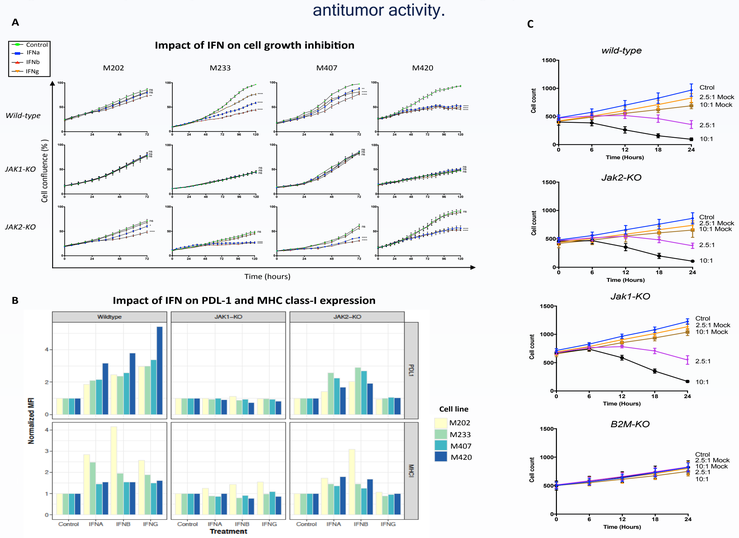

Fig. 1: (A) Human melanoma cell lines showed growth inhibition in response to direct in vitro treatment with interferon alpha, beta, or gamma, whereas the *JAK2-KO* was insensitive specifically to interferon gamma and the *JAK1*-KO was insensitive to all three interferons. (B) The measure of PD-L1 and MHC class I after IFNs stimulation. *JAK1/2* KOs ability to up-regulate both expressions upon IFN-gamma stimulation were dramatically reduced, compared to WT. (C) Parental M202 as well as the *JAK1/2*-KO and *B2M*-KO were co-cultured and recognized by MART-1-specific T cells. There was no difference in *in-vitro* cytotoxicity against *JAK1/2*-KO-MART-1+ melanoma cells compared to the parental, but *B2M*-KO was resistant to killing. Growth curves represent the percent in the confluence of cells over time as measured by IncuCyte continuous live-cell imaging. ns not significant; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. Cytotoxicity assays were conducted by real-time live cell imaging in an IncuCyte ZOOM (Essen Biosciences). M202 cell line were stably transfected with a nuclear localizing RFP (NucLight Red Lentivirus EF1a Reagent, Essen Biosciences) to facilitate cell counts.

The IFN-gamma-induced increased expression of antigen presenting machinery, IFN-gamma signaling and chemokines is lost with *JAK1/2 LoF* mutations

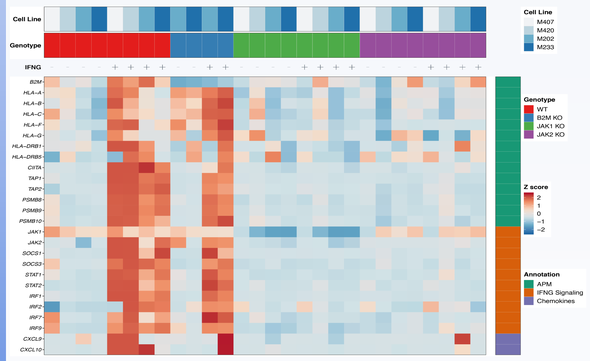

Fig. 2: Immune escape with *JAK* LoF mutations. The IFN-gamma-induced increased expression of antigen presenting machinery, IFN-gamma signaling and chemokines is lost with *JAK1/2 LoF* mutations. Gene expression heatmap from the four groups of melanoma cell lines (x-axis): M407 (NY-ESO-1 positive), M420, M202 (MART-1-positive) and M233 illustrating changes from before/after IFN gamma exposure. Associated cell line, genotype, and INFG treatment are indicated. Gene expression is depicted as the Z score of gene expression (in FPKM) across all samples. Genes (y axis) are ordered by those associated with antigen presentation machinery (APM), IFNG signaling response, and chemokines (shown on the right).

Modeling resistance to PD1-blockade in MC38 mouse model *JAK 1/2* and *B2M* knockout results in resistance to anti-PD-1 therapy

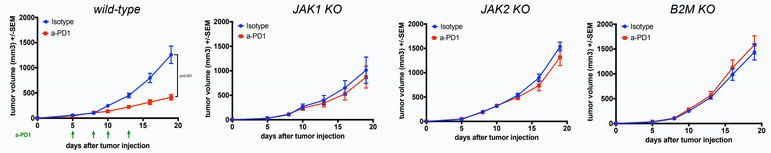

Fig. 3: *JAK 1/2* and *B2M* knockout results in resistance to anti-PD-1 therapy. MC-38 is a model of a highly mutated carcinogen-induced murine cancer, which responds to anti-PD-1 therapy. However, this antitumor response is completely loss when knocking out *JAK1* or *JAK2*, or B2M using CRISPR/Cas9. Tumor growth curves of these cell lines with 5 mice in each group (mean ± SEM) after anti-PD-1 or untreated. The arrow indicates the days of treatment with anti-PD-1 or isotype control was started. p value was determined by unpaired *t* test.

a-PD-1 therapy was unable to increase tumor T-CD8 cells in the *JAK1/2* and *B2M* LoF mutant tumors and predominantly were terminal T-CD8 exhausted

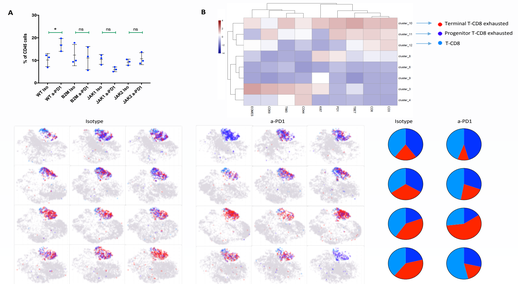

Fig 4: Identification of MC38 tumor T-CD8 immune cell population by CyTOF: (A) Frequency of T-CD8 immune-cell (non-terminal T-CD8 exhausted) displayed on a per-mouse basis with mean-SD unpaired t test (B) Heatmap with the normalized median% for each cluster obtained with FlowSOM on CD8+ T cell markers. Clusters >0.5% were analyzed (C) t-SNE plot of MC38 T-CD8 infiltrating population cells overlaid with color-coded clusters and the differentiation of terminal (red) and progenitor (blue) exhausted T-cells.

Overcome resistance to PD-1 blockade

An intratumoral TLR-9 agonist (SD101) to reverse resistance to a-PD1 in JAK 1/2 LoF resistance tumors

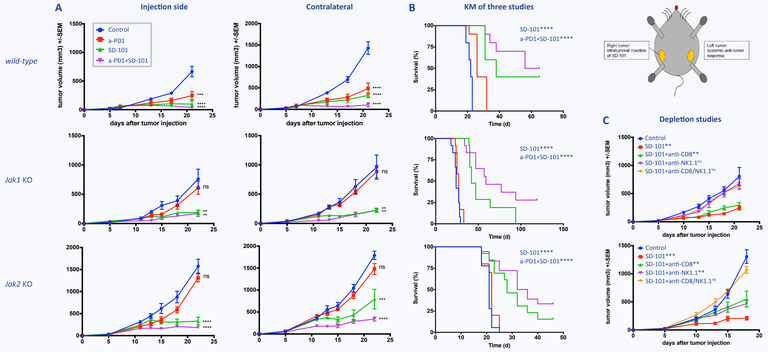

Fig. 5: (A) Effect of intratumoral (i.t) SD-101, TLR-9 agonist, on tumor growth at treat and non-treated "abscopal effect" sites. $3x10^5$ MC38 tumor cells were injected s.c, in both flanks of C57/BL6 mice on day 0. Anti-PD-1 (four doses) treatment started at day 5. Mice were treated with i.p injections of anti-PD-1 or Isotype administered on days 5, 8, 10 and 13. After first anti-PD1 injection, mice started receiving intratumoral injections of 50 ug of SD-101 or CTRL-PBS administered on days 7, 12, 15 and 19. A separate group of mice received SD-101 alone. (B) Long term survival (left flank, n>10 mice per group). Differences in survival were examined using Log-rank (Mantel-Cox) test. (C) Depletion studies with CD8 and NK1.1 antibodies. In (A) and (C) data represented as mean +/- SEM from an n of 5. Dunnett's multiple comparison tests for Control vs. other treatments.

A CD-122 preferential IL-2 pathway agonist (bempegaldesleukin, NKTR-214) to reverse resistance in *B2M* deficient tumors

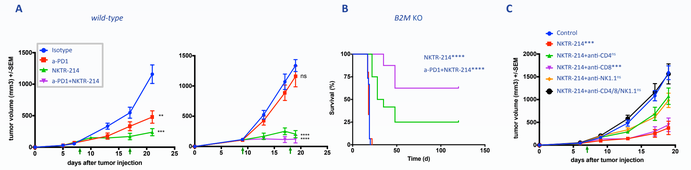

Fig. 6: (A) Effect of bempegaldesleukin-NKTR-214 on tumor growth of MC38 *wildtype* and *B2M* LoF tumors. $3x10^5$ MC38 tumor cells were injected s.c, in left flank of C57/BL6 mice on day 0. Anti-PD-1 (four doses) treatment started at day 5. Mice were treated with bempegaldesleukin (0.8 mg/kg, q9dx2, i.v) on day 8. (B) Long term survival (left flank, n=10 mice per group). Differences in survival were examined using Log-rank (Mantel-Cox) test. (C) Depletion studies with CD4, CD8 and NK1.1 antibodies. In (A) and (C) data represented as mean +/- SEM from an n of 5. Dunnett's multiple comparison tests for Control vs. other treatments.

Conclusions

- *JAK1/2* LoF mutations result in insensitivity to IFN induced antitumor effects, but does not impair T cell recognition and cytotoxicity, while *B2M* LoF results in lack of antigen presentation to T cells and loss of antitumor activity.
- *JAK 1/2* and *B2M* LoF mutations lead to in-vivo resistance to anti-PD-1 therapy.
- *JAK1/2* and *B2M* LoF mutations resistance can be overcome by a TLR9 agonist or a CD-122 preferential IL-2 pathway agonist.

1. Zaretsky JM, et al. N Engl J Med. 2016 Sep 1;375(9):819-29.
2. Shin DS, et al. Cancer Discov. 2017 Feb;7(2):188-201.

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