Overcoming genetically-based resistance mechanisms to PD-1 blockade

Davis Torrejon Castro1,2, Gabriel Abril-Rodriguez1,2, Jennifer Tso1, Ameya Champhekar1, Giulia Parisi1, Gardenia Cheung-Lau3, Tom Wohlwender3, Mykola Onyshchenko1, Beata Berent-Maoz1, Catherine S. Grasso1,2, Begona Comin-Anduix1,3, Siwen Hu-Lieskovsk1,2, Antoni Ribas1,2

1. Department of Medicine, Division of Hematology-Oncology, University of California, Los Angeles, and the Jonsson Comprehensive Cancer Center, Los Angeles, CA 90095, USA
2. Parker Institute for Cancer Immunotherapy, San Francisco, CA 94129, USA
3. Department of Surgery, Division of Surgical-Oncology, and the Jonsson Comprehensive Cancer Center, Los Angeles, CA 90095, USA.

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 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 to characterize the tumor microenvironment. Finally, we tested strategies to overcome resistance mechanisms with SD-101 (TLR-9 agonist) and NKTR-214 (CD-122 biased agonist).

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.

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

Figure 1. In Panel A, human melanoma cell lines showed growth inhibition in response to direct in vitro treatment with interferon alpha, beta, or gamma compared to the control. The JAK1/2 activity was insensitive to anti-interferon gamma and the JAK1/2 was insensitive to all three interferons. In Panel B, the measure of PD-L1 after IFN stimulation. JAK1/2 KO cells were able to regulate both expressions upon IFN-gamma stimulation that were dramatically reduced, compared to WT. In Panel C, Parental M202 as well as the JAK1/2KO and B2M KO were cocultured and recognized by MART1-specific T cells. There was no difference in in-vitro cytotoxicity against JAK1/2KO/MART1 melanoma cells compared to parental, but B2M KO was resistant to killing. Growth curves represent the percent change in the confluence of cells over time as measured by Incucyte continuous live-cell imaging in one of three independent experiments. ns not significant; *p<0.05; **p<0.01; ***p<0.001 for the percent change in growth with the treatment shown at the 72-hour or 120-hour end point compared with the untreated control, with Dunnett’s multiple-comparison correction. Cytotoxicity assays were conducted by real-time live cell imaging in an Incucyte ZOOM (Quesite Biostics). M202 cell line was stably transduced with a nuclear localizing RFP (NucLight Red LentiRFP EF1a Reagent. Eoss Biosciences) to facilitate cell counts.

Figure 2. Immune escape with JAK1/2 LoF mutations. Gene expression heatmaps from the four groups of melanoma cell lines: M202 (WT and JAK1/2-/-ko), M202 SMART-1 (positive) and M233 illustrating changes from before/after IFN-gamma exposure. Red and blue dots represent gene probe sets upregulated and downregulated respectively. Genes over five-fold differentially expressed are indicated on the right side of the first image.

Figure 3. JAK1/2 and B2M knockout results in resistance to anti-PD-1 therapy

Figure 4. Analysis of tumor T CD8 cells populations by Flow. In panel A the percentage of CD8 of PD-1 positive cells. MC38 wild-type treated tumors had increased T cell infiltration relative to untreated (p=0.05). JAK1/2 and B2M KO were unable to increase CD8 after a-PD1 therapy. In panel B the b-PIPE plot of MC38 CD8 infiltrating population cells overlaid with color-coded clusters and the differentiation of exhausted infiltrating T-cells (terminal in red and progenitor in blue).

Figure 5. Effect of intratumoral 0.5 SD-101, TLR-9 agonist, on tumor growth at treated (A) and non-treated (B) sites. 3x10^5 MC38 tumor cells were injected s.c. in both flanks of C57BL/6 mice on day 0. Anti-PD1 (four doses) treatment started at day 5. Mice were treated with IgG injections of anti-PD1 or isotype administered on days 5, 8, 10 and 13. After the 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. Data represented as mean +/- SEM from an n of 8. Dunnett’s multiple comparison test a is not significant, *p=0.05; **p=0.01, ***p=0.001; ****p=0.0001.

NKTR-214, a kinetically engineered IL2 receptor agonist, may overcome resistance mediated by B2M deficient tumors

Figure 6. Effect of NKTR-214 on tumor growth of MC38 wild-type and B2M KO. 3x10^5 MC38 tumor cells were injected s.c. in 8 mice. Each of treated mice on day 0, anti-PD1 (four doses) treatment started at day 5. Mice were treated with NKTR-214 (0.8 mg/kg, q9dx2, 3x10^5) on day 9 when tumor volume reached >100 mm3. Data represented as mean +/- SEM from an n of 4. Dunnett’s multiple comparison test a is not significant, *p=0.05; **p=0.01, ***p=0.001; ****p=0.0001.

Conclusions

We characterize the biological significance of the JAK1/2 and B2M LoF mutations we described in patients samples, which provide strong evidence of the key importance of the IFN-gamma receptor signaling pathway.

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.

JAK1/2 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 new generation IL-2.

REFERENCES


Overcoming genetically-based resistance mechanisms to PD-1 blockade