991. Blockade of the PD-1/PD-L1 Immune Checkpoint Pathway Improves Mortality, Infection Severity, and Fungal Clearance in an Immunosuppressed Murine Model of Invasive Pulmonary Mucormycosis

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Background. Emerging experimental evidence suggests that immune checkpoint inhibitors (ICIs) enhance antifungal immunity. In addition, there is anecdotal evidence of potential benefit of adjunct PD-1 pathway blockade in patients with intractable mucormycosis. However, proof-of-concept data in animal models are lacking. Therefore, we compared the efficacy of PD-1 and PD-L1 inhibition in an immunosuppressed murine model of invasive pulmonary mucormycosis (IPM).

Methods. Female 8-9-week-old BALB/c mice were immunosuppressed with cyclophosphamide (150 mg/kg on days 4 and 1, 100 mg/kg on day 1) and cortisone acetate (300 mg/kg on day 1) and infected intranasally with 50,000 Rhizopus arrhizus spores (clinical isolate Ra-749, day 0). On days 0, +2, +4, and +6, mice received intraperitoneal injections of 250 µg/kg PD-1 or PD-L1 blocking antibodies versus (vs.) 250 µg/kg of the corresponding isotype antibodies (all antibodies from Leinco Technologies). Survival was monitored for 7 days post-infection. Infection severity was scored using the murine sepsis score (MSS: 0 = healthy to 3 = moribund). Fungal burden in lung tissue was determined by an 185 quantitative PCR assay on day 7 or upon death. 20 mice per treatment were assessed in 2 independent experiments.

Results. Control mice with IPM receiving either of the unspecific isotype antibodies developed severe infection (median MSS on day 7: 2.5-3.0) and had a high 7-day mortality (50-55%). Compared to the corresponding isotype control, PD-L1 inhibition showed strong therapeutic benefit, significantly improving morbidity (median MSS = 1.0 vs. 2.5, p = 0.002), 7-day mortality (15% vs. 50%, p = 0.02) and fungal burden (3.66 vs. 27.2k spore equivalents/lung, p < 0.001). In contrast, blockade of PD-L1 modestly yet non-significantly reduced infection severity (median MSS = 2.1 vs. 3.0, p = 0.48), 7-day mortality (35% vs. 55%, p = 0.12), and fungal burden (5.6k vs. 40.7k spore equivalents/lung, p = 0.09) compared to isotype control.

Conclusion. Even without concomitant antifungals, blockade of PD-L1 and to a lesser extent of PD-1 improved mortality, infection severity, and fungal clearance in immunosuppressed mice with IPM. Immune phenotyping studies are in progress to better understand the protective antifungal activity of ICIs in IPM.

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Results.
CRISPRclean treatment of the fully contrived samples increases the fraction of reads that map to the SARS-CoV-2 genome by an average of ~10-fold

**Figure 1:** Schematic of the Jumpcode CRISPRclean protocol

**Figure 2:** CRISPRclean workflow easily integrates into next generation sequencing projects

**Figure 3:** Metatranscriptomics powered by CRISPR-mediated rRNA depletion offers a robust methodology to acquire viral genomic data, microbiome composition, co-infection information, and the transcriptional status of the host immune response in a single workflow.

**Figure 4:** CRISPRclean treatment of the contrived samples increases ~10 fold of reads after CRISPRclean depletion.

**Table 1:** SARS-CoV-2 fraction of total RNA

| SARS CoV-2 fraction of total RNA | % of genome covered at 1X | % of genome covered at 10X |
|----------------------------------|--------------------------|---------------------------|
| 1.0000%                          | 100%                     | 100%                      |
| 0.1000%                          | 100%                     | 100%                      |
| 0.0100%                          | 100%                     | 100%                      |
| 0.0010%                          | 80%                      | 48%                       |
| 0.0001%                          | 30%                      | 7%                        |

Coverage of the SARS-CoV-2 genome at 50 million reads.

**Figure 5:** For the sample containing 0.0001% SARS-CoV-2, (60 viral copies), the number of reads mapping to the SARS-CoV-2 genome increases from ~10,000 reads to ~70,000 reads after CRISPRclean depletion.

**Figure 6:** For the sample containing 0.0001% SARS-CoV-2, (60 viral copies), the number of reads mapping to the SARS-CoV-2 genome increases from ~10,000 reads to ~70,000 reads.

**Conclusion.** CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome.

For the 60 viral copies of SARS-CoV-2 sample, the number of reads mapping to the SARS-CoV-2 genome increases from ~10,000 reads to ~70,000 reads. A similar increase in reads occurs for S. aureus. The percentage of SARS-CoV-2 genome covered at 1X and 10X also increases. Similar results were achieved even after downsampling the datasets to 5M reads. There is a ~4 fold increase in bacterial species detection in these stool samples after CRISPRclean treatment. Percentage of SARS-CoV-2 genome covered at 1X and 10X increases as a result of rRNA depletion.

**Figure 7:** For the sample containing 0.0001% SARS-CoV-2, (60 viral copies), the number of reads mapping to the SARS-CoV-2 genome increases from ~10,000 reads to ~70,000 reads.

**Figure 8:** CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome.

**Figure 9:** For the 60 viral copies of SARS-CoV-2 sample, the number of reads mapping to the SARS-CoV-2 genome increases from ~10,000 reads to ~70,000 reads.

**Figure 10:** CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome.

**Figure 11:** CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome.

**Figure 12:** CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome.

**Figure 13:** CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome.

**Figure 14:** CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome.

**Figure 15:** CRISPRclean treatment of the contrived samples increases ~10 fold of reads that map to the SARS-CoV-2 genome.