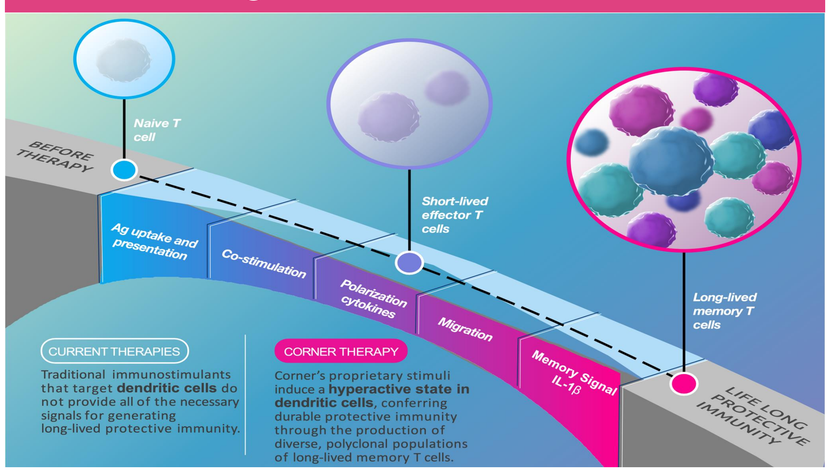
A Novel Vaccine Adjuvant Activates NLRP3 Inflammasomes in Living Dendritic Cells to Stimulate Robust T Cell Immunity

Stephan Matissek, Saisha Nalawade, Carolyn Macfarlane, Veronica Komoroski, Jonathan Chow, Sivan Elloul, Jonathan Kagan

CORNER THERAPEUTICS

Visit Our Website

Memory T cell responses require five activation signals from dendritic cells



- Dendritic cells (DCs) are the only cells that can stimulate new T cell responses to cancer and infection by providing multiple signals: antigen in the context of MHC molecules, costimulatory proteins, and inflammatory cytokines. In order to elicit a durable T-cell response, additional factors are needed including secretion of IL-1β and the ability of DCs to migrate.
- Hyperactivation is a cell state where living DCs hypermigrate to lymph nodes and secrete IL-1β in the absence of pyroptosis, enabling other critical functions as antigen presenting cells. Hyperactivation has been shown to induce the polarization of T cells to a Th1 phenotype, demonstrated to be beneficial for combating viral infection and cancer.
- **Corner Therapeutics has developed CRN-1001, a novel combination of TLR7/8 agonist (R848) and our proprietary hyperactivating lipid (CT009), for clinical use. CRN-1001 hyperactivates DCs and promotes durable T cell-mediated immunity.**

➢ The study aims to demonstrate the impact of hyperactivation on the mRNA expression profile of human moDCs and provides evidence that hyperactivated moDCs exhibit enhanced migration, increased IL-1β secretion, and improved T cell activation

CRN-1001 induces DC hyperactivation, leading to expression of cytokines and activation markers

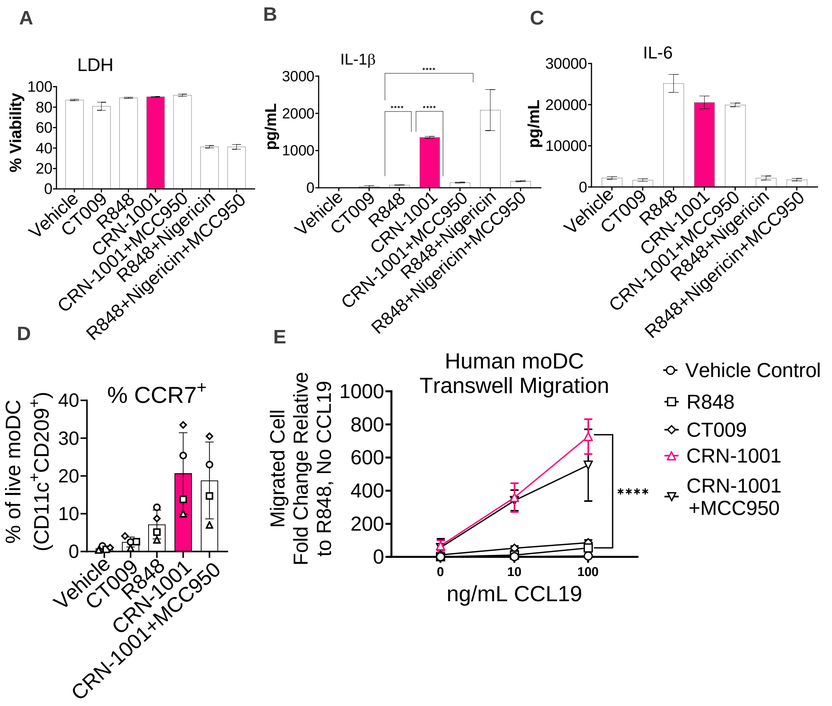


Figure 1: Human monocyte derived dendritic cells **(**moDCs) were treated for 24 hours with R848 and CT009 individually or in combination. To induce pyroptosis, cells were primed with R848 for 3 hours prior to stimulation with Nigericin. MCC950, a selective inhibitor of NLRP3, was used to understand inflammasome dependency of the respective treatment (A) One day after indicated moDC treatments, LDH was measured to determine viability of cells. Cell culture supernatant and ELISA was used to measure (B) IL-1β and (C) IL-6. (D) Flow staining was performed to assess expression of lymph node-homing chemokine receptor CCR7. (E) Monocyte-derived dendritic cells (moDCs) were tested for migration using a transwell assay after being treated with R848 and/or CT009. Cells that migrated towards varying concentrations of CCL19, located in the basal chamber, were counted.

When treated with CRN-1001, moDCs secrete IL-1β in the absence of pyroptotic cell death, which is a needed signal for memory T cell formation. This effect is inflammasome dependent due to the inhibition of IL-1β by MCC950. Additionally, CRN-1001 treatment enhances ability of moDCs to migrate towards CCL19, a chemokine crucial for DC migration to lymphoid tissue.

Preparation of differentially activated DCs for RNAseq Analysis

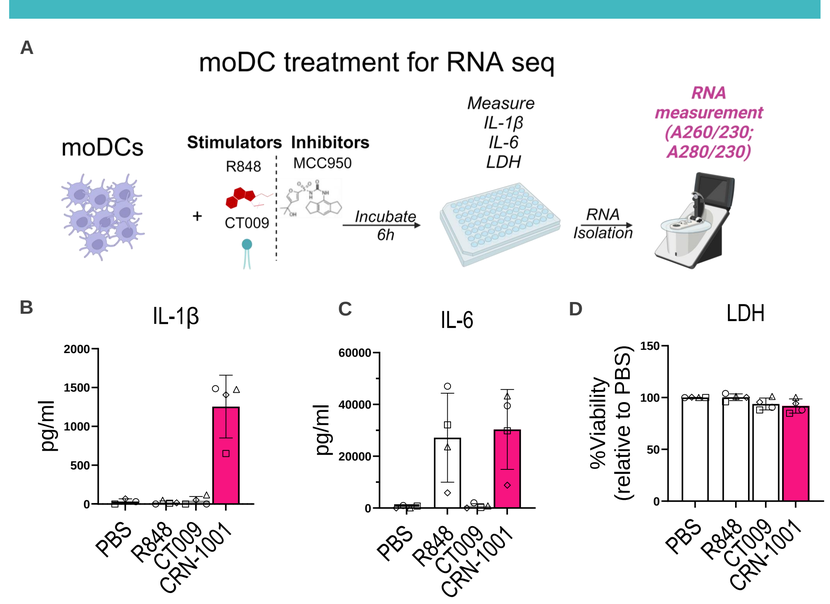


Figure 2: moDCs were treated for 6 hours with R848 and CT009 individually or in combination. (A) Schematic depicts treatment procedure of moDCs for RNA seq library preparation. moDCs were treated with different stimuli for 6h prior to collection of supernatant for (B) IL-1β, (C) IL-6 and (D) lactate dehydrogenase (LDH) analysis. Cell lysate was collected for RNA analysis.

Treatment of moDCs with CRN-1001 for 6h led to secretion of IL-1β while maintaining IL-6 expression and viability. Since hyperactive state was confirmed, RNA was isolated from those cells for assembly of RNA seq library.

CRN-1001 induces a unique transcriptional program in human DCs

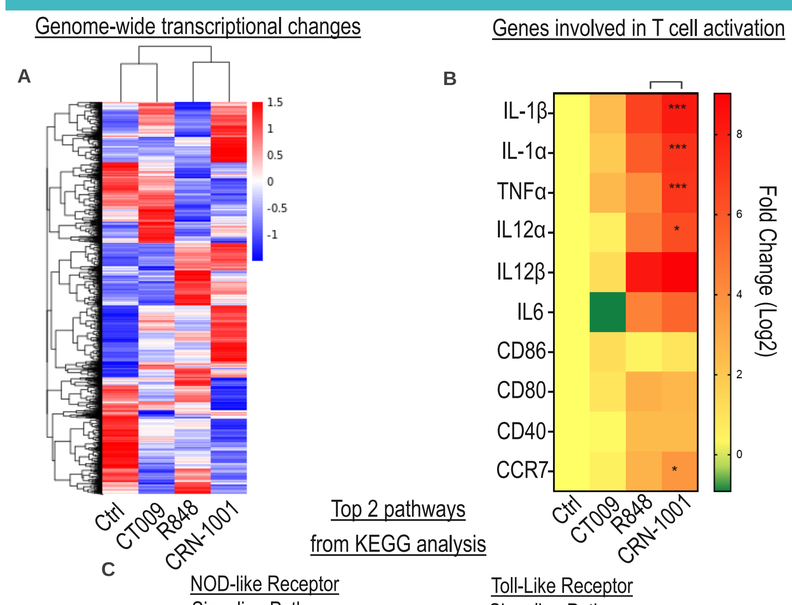


Figure 3: Shift in gene expression profile after moDCs were treated with CRN-1001. Heatmaps show in (A) differences in gene expression in log2 fold change after treatment, (red: upregulated, blue: downregulated) and in (B) genes that are involved in T cell activation. Significant changes in gene expression between CRN-1001 vs R848 are indicated by asterisks. (C) Heatmap showing top 10 genes that were differentially regulated based on results from KEGG pathway analysis. Significant changes in gene expression between CRN-1001 vs R848 are indicated with asterisks. Colors indicate log2 fold change in gene expression (red: upregulated, yellow: no change, green: downregulated). *** p<0.0001 , ** p<0.001, * p<0.05. Statistical analysis was performed using DESeq2. P-values were adjusted using the Benjamini and Hochberg's approach.

Six-hour treatment with CT009, R848 and CRN-1001 each led to unique gene expression patterns, highlighting the distinct cell state of hyperactivation. Results aligned with previously known features of hyperactivation such as increased expression of IL-1β and CCR7. Finally, NOD-like receptor and Toll-like receptor signaling pathways were the most affected by CRN-1001, with a variety of genes, including cytokines, transcription factors, and apoptosis regulators, showing the greatest differential regulation

CRN-1001 increases expression of genes involved in Th1 T cell polarization

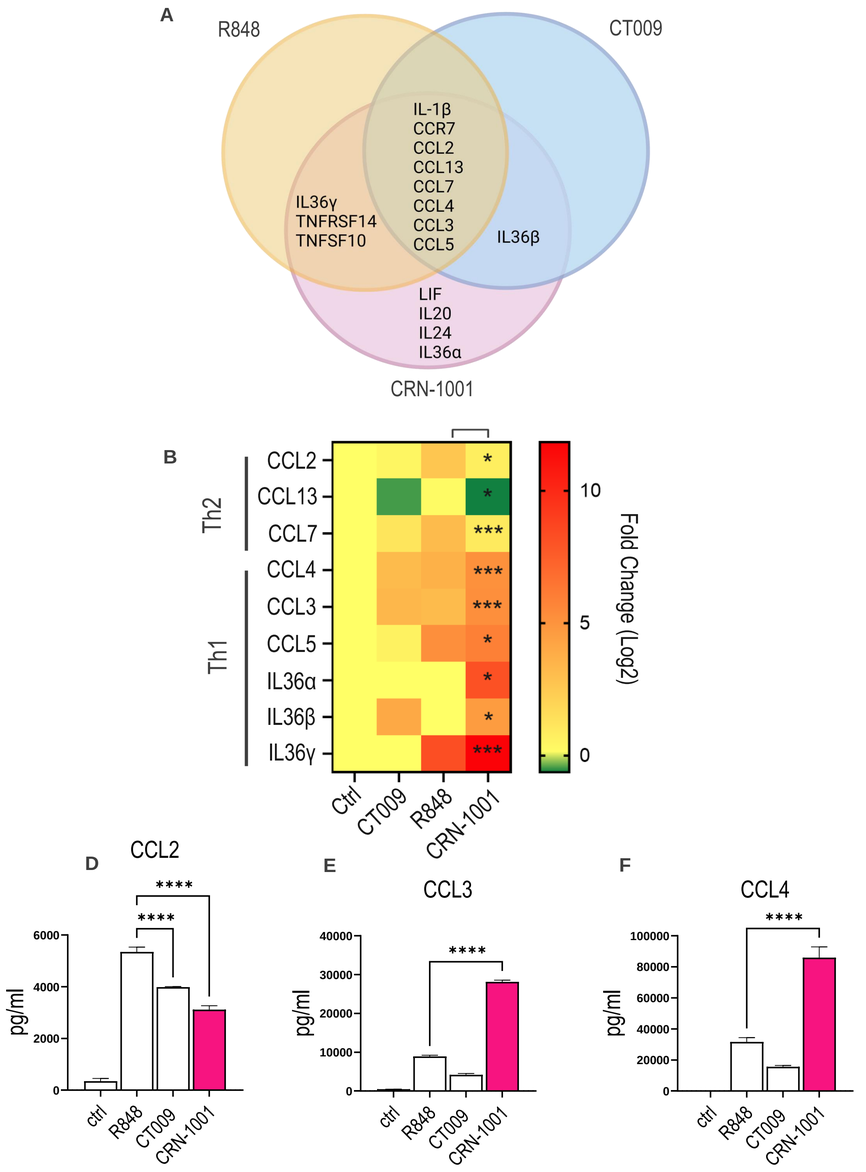


Figure 4: CRN-1001 treatment leads to increased expression of T cell stimulating cytokines. Venn diagram showing (A) example genes that are shared or exclusively expressed. All genes are significantly upregulated under hyperactive condition in comparison to R848. (C) Heatmap showing log2 fold change in comparison to ctrl for genes that are listed in venn diagram and related to Th1 and Th2 responses (B). To verify RNAseq data moDCs were treated with different stimuli for 6h prior to collection of supernatant, that was analyzed for (D) CCL2, (E) CCL3 and (F) CCL4 using ELISA. *** p<0.0001 , ** p<0.001, * p<0.05. For statistical analysis of RNA seq data DESeq2 was used. P-values were adjusted using the Benjamini and Hochberg's approach. One-way ANOVA was used for statistical analysis of ELISA data.

Venn analysis identified additional genes affected by hyperactivity that are involved in cytokine signaling and T cell regulation. Notably, chemokines involved in Th2 signaling were downregulated upon hyperactivation, while Th1-related chemokines and cytokines were upregulated. Upregulation of Th1 genes and downregulation of Th2 genes was evaluated at the protein level by ELISA (CCL2, CCL3 and CCL4) and confirmed mRNA results, suggesting that hyperactivation plays a role in promoting Th1 T cell polarization.

NLRP3 mediates memory TH1 T cell activation by hyperactive DCs

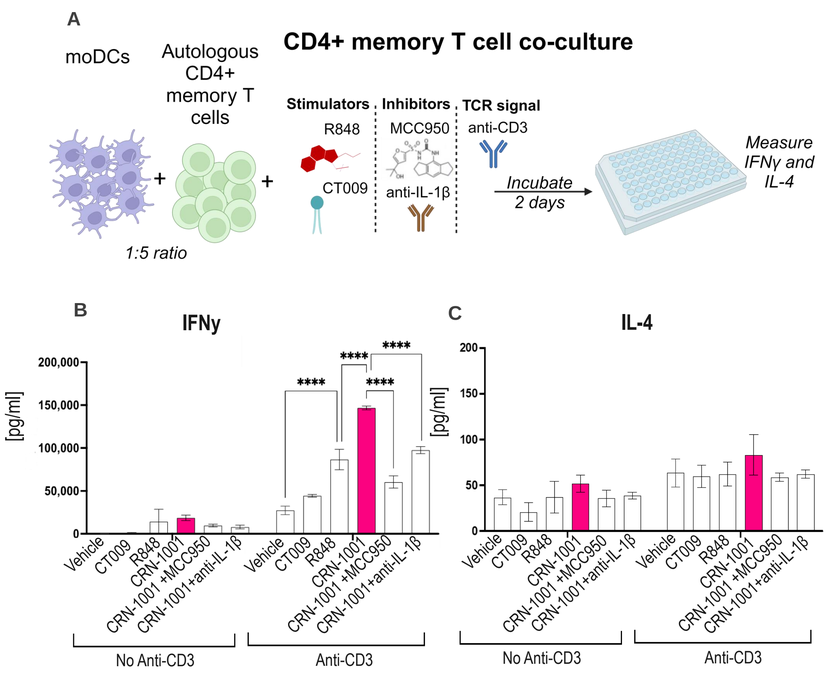


Figure 5: Human moDC were co-cultured with CD4+ T cells to study downstream effects of hyperactivation. (A) Schematic of the co-culture setup to assess memory CD4+ T cell responses in the presence of hyperactivated moDCs. moDCs were co-cultured with autologous memory CD4+ T cells stimulated with and without 0.1ng/mL anti-CD3. (B) To investigate the role of the inflammasome in hyperactivation-mediated IFNγ or IL-4 secretion, MCC950 was used. Additionally, to demonstrate the necessity of IL-1β in this process, anti-IL-1β antibodies were utilized. (B) IFNγ and (C) IL-4 were measured 2 days after coculture setup.

Memory CD4+ T cells cultured with hyperactivated moDCs increase secretion of IFNγ, suggesting an enhanced Th1 response. In contrast IL-4 levels remain unchanged, indicating that CRN-1001-treated moDCs do not drive Th2 responses. The increased IFNγ production is partially IL-1β dependent, as indicated by conditions containing the inflammasome inhibitor MCC950 or anti-IL-1β blocking antibody.

NLRP3 mediates antigen specific CD8+ T cell activation by hyperactive DCs

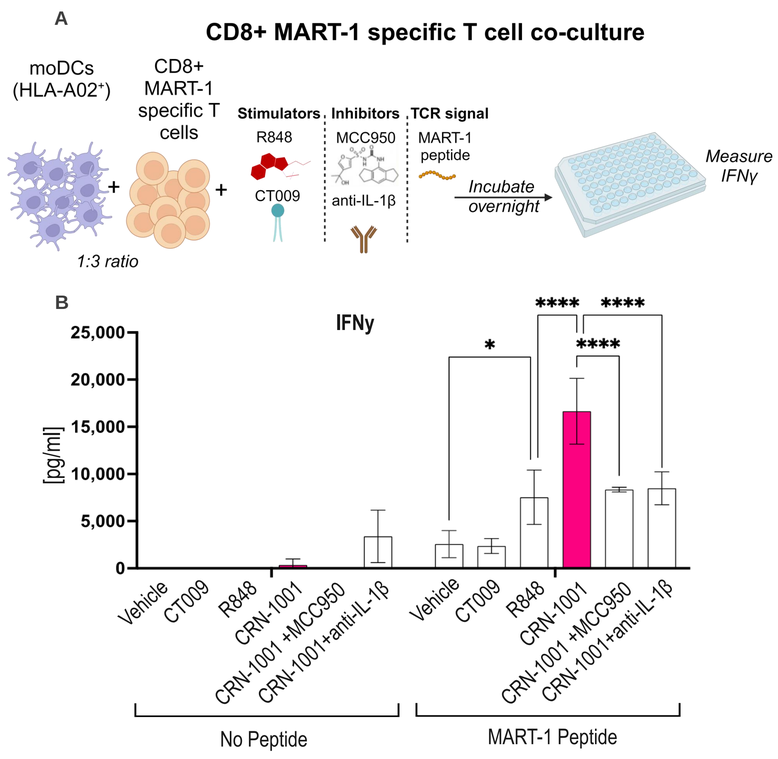
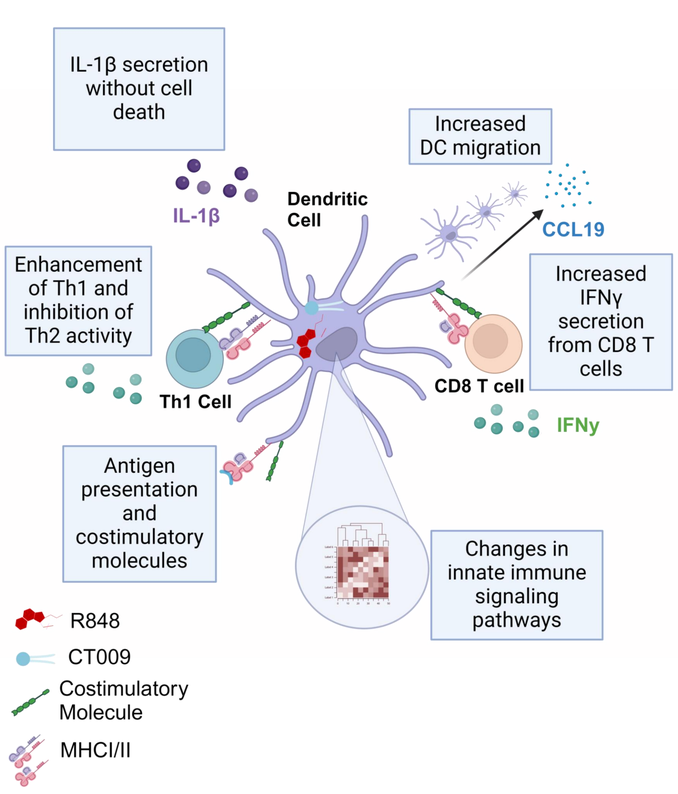


Figure 6: Human moDC were co-cultured with CD8+ T cells to study downstream effects of hyperactivation. (A) Schematic depicts the co-culture setup to assess antigen-specific CD8+ T cell responses in the presence of hyperactivated moDCs. HLA-A02+ moDCs were cocultured with MART-1 specific CD8+ T cells stimulated with MART-1 peptide. (B) moDCs and CD8+ T cells were cocultured in indicated condition for 1 day. Inflammasome or IL-1β dependent secretion of IFNγ was evaluated by using the inflammasome inhibitor MCC950 and anti-IL-1β antibody. Secreted IFNγ was measured by collecting supernatant and performing Lumit IFNγ Immunoassay.

MART-1-specific CD8+ T cells cultured with hyperactivated moDCs showed significantly increased secretion of IFNγ in an antigen specific manner. The increase in IFNγ production is dependent on inflammasome activity and IL-1β secretion from hyperactivated moDCs.

Hyperactivated DCs provide functions needed for durable T cell responses