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POSTER ABSTRACTS

622.LYMPHOMAS: TRANSLATIONAL-NON-GENETIC

Pathobiology and Targeting of CD38 in Cutaneous T-Cell Lymphoma

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Introduction: Cutaneous T-cell lymphoma (CTCL) is a malignancy of mature CD4+ T-cells that primarily affects the skin. Despite a wide variety of topical and systemic therapies for patients, CTCL remains difficult to treat. Because of its high likelihood of relapse and resistance, new therapeutic approaches are needed. We recently showed strong CD38 expression on neoplastic T-cells from patients with mature T-cell neoplasms, including CTCL. In this study, we evaluated i) the role of CD38 in CTCL pathogenesis ii) strategies to enhance CD38 expression in CTCL and iii) tested efficacy of combination therapy *in vivo*.
Methods: Skin and peripheral blood samples from patients and healthy donors were evaluated for CD38 expression by immunohistochemistry (N=7), microarray (N=82), single-cell RNA-sequencing (N=11), and flow cytometry (N=14). To investigate the role of CD38 in CTCL, we generated CD38 knockout cell lines (H9, HH, and HuT-78) using CRISPR/Cas9-mediated genomic deletion. The CD38 wild-type (CD38 ^{WT}) and knockout (CD38 ^{KO}) cells were purified, cultured, and used for downstream functional analysis. We evaluated the lines for growth, metabolic capacities (XFe24 Seahorse assay), and engraftment potential *in vivo*. For *in vivo* therapeutic studies, we transduced firefly-luciferase gene into H9 CD38 ^{WT} and CD38 ^{KO} cells, and engrafted them intravenously into immunodeficient NOD Rag $^{-/-}\gamma c^{-/-}$ mice. Mice were treated with anti-CD38 antibody daratumumab or IgG control and disease progression was monitored over time using an in vivo imaging system. For combination studies, we evaluated CD38 expression by flow cytometry in H9 cells treated with increasing doses of HDAC inhibitor, panobinostat (5nM, 10nM, 25nM) for 24, 48, and 72 hours. Next, we tested co-treatment with panobinostat and daratumumab *in vivo* in mice engrafted with H9 CD38 ^{WT} cells with four treatment groups (vehicle/IgG, panobinostat/IgG, vehicle/daratumumab, and panobinostat/daratumumab).
Results: Patient skin biopsies showed increased CD38 expression at both protein and RNA levels (log fold change 4.8; $p<0.0001$ by Mann-Whitney test). Across nine days of tracking under standard cell culture conditions, CD38 ^{KO} CTCL cells showed minimal growth differences compared to CD38 ^{WT} ($p=0.22$ by linear regression). However, CD38 ^{KO} CTCL cells showed significantly increased basal and maximum oxygen consumption rates (OCR) via seahorse assay when compared to CD38 ^{WT} cells (basal OCR 2.26 fold increase, $p<0.0001$; max OCR 1.83 fold increase, $p<0.0001$ by Mann-Whitney test). Using IVIS luminescent imaging, we found significantly increased *in vivo* growth of CD38 ^{KO} CTCL cells compared to CD38 ^{WT} cells (CD38 ^{WT}=2.2e8 photons/sec average total flux, N=7; CD38 ^{KO}=1e9 photons/sec, N=5; $p=0.003$ by Mann-Whitney test). These data suggest that the loss of CD38 in CTCL cells can accelerate the development of tumors in mice. In order to study the effectiveness of a targeted drug against CD38 in CTCL, we used anti-CD38 antibody daratumumab in our H9 xenograft model. Daratumumab treatment had a significant therapeutic effect on CD38 ^{WT} tumors compared to isotype control, reducing tumor burden by 84% (daratumumab average total flux=1.4e7 photons/sec, N=4; IgG average total flux=9.0e7 photons/sec, N=3; $p=0.0002$). Additionally, we explored methods to increase CD38 expression on CTCL cells. We found that panobinostat significantly increased CD38 expression on H9 CTCL cells in a dose dependent manner across multiple time points (max 85% increase in CD38 expression with 25nM panobinostat vs. DMSO at 72 hours; $p<0.0001$ by 2way ANOVA). *In vivo* testing demonstrated a statistically significant improved survival benefit in mice treated with the combination of panobinostat and

daratumumab compared to mice that received daratumumab alone (vehicle median survival 23 days, N=4; daratumumab alone median survival 32 days N=4; panobinostat alone median survival 27.5 days, N=4; combination median survival 39 days, N=4; p=0.01 by log-rank test). The prolonged average survival benefit amounted to approximately one quarter of their entire overall lifespan.
Conclusion: Our studies demonstrate strong evidence for further study into how CD38 regulates the growth and survival of CTCL cells. Our data also provide preliminary evidence for the clinical usefulness of combination therapies that increase CD38 expression to enhance tumor cell immunotherapeutic targeting.

Disclosures Porcu: *Kyowa, Daiichi, Viracta, Dren Bio, Innate Pharma, Ono:* Honoraria; *Kyowa, Daiichi, Viracta, Dren Bio, Innate Pharma:* Consultancy; *Ono:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Dren-Bio, ADCT, Lilly-Loxo, Viracta, Innate Pharma:* Membership on an entity's Board of Directors or advisory committees; *BioGene:* Membership on an entity's Board of Directors or advisory committees; *Kyowa:* Consultancy; *Kymera:* Membership on an entity's Board of Directors or advisory committees; *Teva:* Research Funding; *Innate Pharma:* Research Funding.

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