55th Annual Midwest Student Biomedical Research Forum

Saturday, March 2, 2024

ROOM 3047

10:15 a.m. **O-20** EFFICACY OF MRI/TRANSRECTAL ULTRASOUND BIOPSY VS. STANDARD MAPPING BIOPSY IN DETECTING CLINICALLY SIGNIFICANT PROSTATE CANCER
Presenter: Aidan Gaertner, Creighton University

10:30 a.m. **O-25** CCR4 MUTATIONS DYSREGULATE T-HELPER DIFFERENTIATION AND PROMOTE LYMPHOMAGENESIS
Presenter: Dylan Jochum, UNMC

10:45 a.m. **O-33** PANCREATIC CANCER AND T-CELL CROSSTALK-INDUCED MUC4 EXPRESSION ATTENUATES T-CELL-MEDIATED RESPONSE
Presenter: Xiaoqi Li, UNMC

11:00 a.m. **O-42** DIRECT NEOANTIGEN IDENTIFICATION IN PANCREATIC ADENOCARCINOMA
Presenter: William Miklavcic, UNMC

11:15 a.m. **O-68** LONG-TERM SURVIVAL RATES OF BURKITT LYMPHOMA PATIENTS WITH HIV – A NATIONAL CANCER DATABASE (NCDB) STUDY
Presenter: Ashley Tuin, Creighton University

11:30 a.m. Break

EFFICACY OF MRI/TRANSRECTAL ULTRASOUND BIOPSY VS. STANDARD MAPPING BIOPSY IN DETECTING CLINICALLY SIGNIFICANT PROSTATE CANCER

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Background:
UroNav is a diagnostic tool used to improve detection of clinically significant prostate cancer. The technology allows fusion of a multiparametric prostate MRI with a transrectal prostate ultrasound (MRI/TRUS) which improves accuracy of prostate biopsies. Minnesota Urology was an early adopter of this technology. Besides improving detection of cancer, the goal was to avoid performing saturation biopsies and decrease side effects of multiple biopsies This study aims to evaluate the utility of the Uronav (Type of MRI/TRUS) vs. standard saturation biopsies performed by Minnesota Urology over a five year period of time to see if the UroNav provides benefit in detecting not only prostate cancer but clinically significant prostate cancer at a higher rate.

Methods:
The Minnesota urology database was analyzed from 2015 to the present. Over a five year period 5307 prostate biopsy results were retrospectively analyzed. The percent detection of prostate cancer was evaluated in three patient groups: patients that underwent standard mapping biopsies, UroNav plus standard mapping biopsies and UroNav only targeted biopsies. Percent of biopsies done that had a gleason score >= 7 (used as the cutoff for determining clinically significant prostate cancer) was obtained. Kaplan-Meier, Cox Proportional Hazards test, ANOVA and Chi-Square tests were performed. Data was analyzed using SPSS version 27 and statistical significance was set at $\alpha = 0.05$.

Results:
5,307 biopsies were analyzed over a five year period performed by the Minnesota Urology PA. 3,626 patients underwent a standard mapping biopsy, 1,472 patients a UroNav with standard biopsy, and 209 patients a UroNav alone. Prostate cancer was detected at a higher rate when UroNav + standard mapping was used (63.59%) vs. standard mapping alone (45.67%) vs. UroNav alone (31%) (P value <0.001). Out of the positive biopsies, the Uronav + standard mapping biopsy technique had a higher rate of detecting clinically significant prostate cancer vs standard mapping biopsy alone or UroNav alone (30.98% vs 26.34% vs 21.05%) respectively (P Value <0.05).

Conclusion: UroNav prostate biopsy had an increased accuracy and rate of detecting clinically significant prostate cancer. This provides a diagnostic advantage over regular standard mapping biopsy. It also is better for the general health of patients who will undergo less repeat prostate biopsies which are prone to infection. With this data the UroNav is being adapted as the standard of care for the Minnesota Urology group.

CCR4 MUTATIONS DYSREGULATE T-HELPER DIFFERENTIATION AND PROMOTE LYMPHOMAGENESIS

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Background: Peripheral T-cell lymphoma (PTCL) includes a diverse group of post-thymic T-cell neoplasms; approximately 40% are designated as PTCL-not otherwise specified (PTCL-NOS), remaining unclassifiable within World Health Organization (WHO) and International Consensus Classification (ICC) PTCL entities. Using gene expression profiling (GEP), we identified two novel molecular subgroups (i.e., PTCL-GATA3 and PTCL-TBX21) within PTCL-NOS cases designated with distinct T-helper (T_H) transcriptional programs and clinicopathological features. The pathogenesis of PTCL-NOS subgroups is still poorly understood. PTCL-GATA3 represents 40% of PTCL-NOS and is defined by its high expression of GATA3, the master transcriptional regulator of T_H2-differentiation. PTCL-GATA3 is clinically more aggressive than other PTCL entities. High-throughput genomic analysis revealed a high frequency of genetic aberration in TP53 (~49%), PTEN (~29%), and CCR4 (~15%). A high frequency of mutations occurs in the cytoplasmic domain of C-C Motif Chemokine Receptor 4 (CCR4), a G-protein coupled receptor and GATA3 transcriptional target, resulting in gain-of-function traits. Data suggests these mutations are independent of *TP53* mutation, another widespread driver mutation in PTCL-GATA3, pointing to the presence of an alternative a novel mechanism. The mutant cases exhibit increased overall CCR4 expression compared to wild-type, linked to slower endocytosis post-ligand stimulation, implying a selective pressure favoring the role of CCR4 mutants in T_H2-cell lymphomagenesis.
Significance of Problem: CCR4 mutations are ubiquitous across numerous lymphoma types, including but not limited to, adult T-cell leukemia/lymphoma and peripheral T-cell lymphoma. CCR4 mutations correlate with increased cell migration and proliferation, ultimately driving lymphomagenesis, although the mechanism(s) for this pathway is not well understood. Additionally, CCR4 mutation plays a major role in T-cell receptor (TCR) activation and signaling, as well as dysregulating T-helper cell differentiation. Thus, this study seeks to elucidate the mechanisms by which CCR4 mutations drive these tumorigenic pathways driving lymphomagenesis and shed light on CCR4 as a compelling therapeutic target.
Hypothesis, Problem, or Question: This study is investigating the mechanism by which mutations in CCR4 enhance T-cell lymphomagenesis and T-helper differentiation through aberrant T-cell activation and TCR signaling.
Experimental Design: Clinical outcome data were assessed with the Kaplan-Meier method. Mutational analysis of driver genes was generated from whole exome sequencing. In vitro analysis of CCR4 cytoplasmic domain mutants utilized healthy-donor CD4+ T-cells or the CD4+ Jurkat T-cell line. TCR signaling was determined by Phosflow™ (BD Biosciences) flow cytometry analysis of p-Lck, p-SLP76, and p-ZAP70. Transwell assays evaluated cell migration with ectopic CCR4 expression in response to rCCL17 and rCCL22 (R&D Systems). Polarization studies were performed by removing the cells from normal conditional media for 24 hours, then treating with either T_H1- or T_H2-associated cytokines, or normal conditioning media. Cell viability, GATA3, TBX21 expression, and phospho-AKT protein levels were measured using PrestoBlue™ (ThermoFisher), True-Nuclear™ Buffer Set (BioLegend), and Western blotting, respectively. Surface plasmon resonance determined the binding affinity of wild-type and mutant CCR4 in response to CCL17 and CCL22. Protein-antibody docking studies using Schrödinger's BioLuminate, where we docked anti-CCR4 monoclonal antibody, Mogamulizumab, into the AlphaFold structures of CCR4-WT and mutants to study their effect on antibody binding. Significance was determined with p-values < 0.05 after corrections for false discoveries.
Results/Data: Ectopic expression of *CCR4* cytoplasmic domain mutants led to an observed TCR activation, AKT/mTOR signaling, and migration upon CCL22 stimulation in Jurkat cells compared to control vectors. When expressed in CD4+ T-cells, these cells did not require CD3/CD28 for growth, bypassing CD3 and CD28 ligation for aberrant T-cell activation. Proliferation and TBX21 expression in T_H1-conditional media were inhibited compared to WT and empty vector, indicating a potential T_H1-differential block. However, these cells exhibited similar proliferation rates in both T_H2-conditional media and normal media. Surface plasmon resonance revealed CCL22 had a higher binding affinity compared to CCL17 in all CCR4 proteins, as previous studies suggested. In addition, all mutant proteins bound tighter (K_D by 1.5- to 3-fold) to both ligands compared to wild-type. CCR4-CDKO was not able to generate any binding data with CCL17 and had a lower binding affinity with CCL22, suggesting that retaining some of the cytoplasmic domain is required for efficient ligand binding. Mutant proteins (L325fs, Q330X, and Q349X) also bound tighter to Mogamulizumab compared to wild-type. Interestingly, Q330X bound to Mogamulizumab in an altered orientation than compared to WT, an observation also seen with CDKO, although this still resulted in an increased binding affinity with its ligands.
Conclusions: *CCR4* mutations have a key role in PTCL-GATA3 pathogenesis by dysregulating T_H-differentiation and promoting cell growth in the absence of CD3 or CD28 ligation. Additionally, CCL22 stimulation increases TCR signaling through aberrant phosphorylation of Lck, ZAP70, and SLP76. Ongoing research, using proximity labeling and mass spectrometry, will explore how *CCR4* mutations cause abnormal T-cell activation and signaling, involving relevant proteins. CCR4 mutant proteins demonstrated an increased binding affinity towards its known ligands, CCL17 and CCL22, as well as Mogamulizumab, highlighting CCR4 and its mutations as promising therapeutic targets in peripheral T-cell lymphoma. The data herein supports the novel role of mutant CCR4 in enhancing T-cell lymphomagenesis and T-helper differentiation, emphasizing these mutants as a therapeutic target with Mogamulizumab.

PANCREATIC CANCER AND T-CELL CROSSTALK-INDUCED MUC4 EXPRESSION ATTENUATES T-CELL-MEDIATED RESPONSE

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Background: While T-cell-based immunotherapy emerged as a promising tool for cancer management, clinical trials and translational studies revealed that cancer cells develop resistance by modulating tumor microenvironment and cell-intrinsic mechanisms. Pancreatic cancer (PC), consisting of dense stroma, immunosuppressive environment, and aberrant mucin expression, has a dismal survival rate and responds poorly to immunotherapies. MUC4, a member of the mucin family, has been reported to block lymphokine-activated killer cells and induce apoptosis of cytotoxic T-cells, suggesting its possible role in immune modulation. Meanwhile, individual cytokines, such as interferon (IFN)-γ and IL-17, induced mucin expression in PC cell lines. However, how the T-cell secretome influences MUC4 expression in PC and its role in modulating the T-cell response is poorly investigated.

Significance of Problem: Understanding the regulation of MUC4 upon T-cell presence and its role in T-cell-mediated cytotoxicity resistance could facilitate the PC patient treatment stratification when giving immunotherapy, improving immunotherapy efficiency and patient outcomes. It could also provide new directions to develop a combination therapy against MUC4-expressing cancers.

Hypothesis: Cancer-T-cell crosstalk upregulates MUC4 expression and attenuates T-cell responses

Experimental Design: The scRNA-seq data analysis and primary T-cell conditioned media (CM) were utilized to investigate the MUC4 and T-cell crosstalk. T-cell CM-treated murine PC cell line (KCT-3266) was analyzed by RNA-seq analysis followed by MUC4 silencing studies to investigate its role in evading T-cell response. The subcutaneous murine model was utilized to investigate the influence of MUC4 KO on immune cells and cytotoxic cell infiltration. The RNA isolated from tumor tissues from $Kras^{G12D/+,}$ $Trp53^{R127H/+}$, Pdx-1-Cre (KPC), and Muc4 knockout (KPMC) murine models were used for PanCancer immune profiling.

Results: The scRNA-seq analysis suggests that intratumoral T-cells positively correlate with MUC4 expression in PC patients. The activated T-cell CM consisting of predominantly IL-2, IFN-γ, and TNF-α cytokines, significantly induce the expression of MUC4 transcriptionally and translationally in both human (SW1990 and COLO357) and murine (KCT-3248 and KCT3266) PC cell lines. RNA-seq analysis from T-cell CM-treated cancer cells revealed that T-cell secretome induced the pathways related to cancer cell death. The CRISPR knockout of MUC4 in PC cells showed increased expression of cleaved caspase-3 after T-cell CM treatment, indicating that MUC4 plays a protective role against the T-cell CM-mediated killing. Subcutaneous implantation of MUC4 proficient and deficient PC cells on the flanks of C57BL/6 immunocompetent mice demonstrated a significantly higher infiltration of CD3 positive and cytotoxic T-cell infiltration in the MUC4 deficient tumors, resulting in significantly lower tumor weight. The PanCancer immune profiling also showed that depletion of Muc4 increases the T-cells and cytotoxic T-cell signaling scores in the KPMC tumor tissues compared to KPC.

Conclusion. T-cell secretome induces MUC4 expression in pancreatic cancer cells, and the increased MUC4 expression reduces T-cell infiltration and protects PC cells against T-cell-mediated killing.

DIRECT NEOANTIGEN IDENTIFICATION IN PANCREATIC ADENOCARICINOMA

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Background: Pancreatic adenocarcinoma (PDAC) is an aggressive cancer with a 12% 5-year average survival rate. Mutations that elicit immunogenic responses contribute to the dismal PDAC statistics, resulting in failed CD8+ T-cell recruitment and attenuated cytotoxic response. The relative absence of neoantigens in PDAC, the mutated proteins that elicit an immune response when presented by the Major Histocompatibility Complex (MHC) system, strongly limit efficacy of CAR-T-cell and immune checkpoint blockade therapeutics. Therefore, successful PDAC neoantigen identification and characterization would unlock novel therapeutic avenues. Neoantigens presented to circulating CD8+ T-cells from MHC class I are 8-12 amino acids in length, lending inherent identification difficulty. Further, heterogeneity at the MHC locus results in variations of MHC allele binding affinities for specific neoantigens, resulting in neoantigens being unique from patient to patient. Clinically significant neoantigens can be predicted through genomic sequencing of the patient's excised tumor followed by *in-silico* algorithms which assess MHC binding affinity and potential CD8+ T-cell reactivity. Using these methods, the Balachandran lab has demonstrated how 'high-quality' neoantigens identified from this pipeline induce specific CD8+ T-cell responses in 50% of patients vaccinated with mRNA encoding these neoantigens. Yet, with only half of patients responding to one or more of the 20 predicted neoantigens identified, these *in-silico* analyses alone are insufficient to successfully identify all clinically relevant neoantigens.

Hypothesis: *We hypothesize direct identification of PDAC tumor neoantigens will expose previously undiscovered neoantigens, allowing for identification of common neoantigens presented by specific MHC alleles, with broad-spectrum applicability to all PDAC patients with MHC allele similarity.*

Methods: We have performed 36 MHC class I isolations from nine PDAC donors. To isolate one batch of MHC class I peptides, 8 grams of PDAC tumor tissue with over 70% tumor cellularity was homogenized using liquid nitrogen and subsequent cell lysis buffer incubation. The membrane fraction from the cell lysate was isolated using ultracentrifugation and applied to an Affigel-10 column coupled with pan-anti-human MHC class I antibody (W6/32). After 35 iterative washes with various salt gradients, MHCs and their associated peptides were eluted using acid elution, and the associated peptide sequences further purified through c18 column cleanup. Eluted peptides were analyzed with tandem MS/MS with spectra identification through the PEAKS software. Whole Exome Sequencing (WES) of PDAC tumor tissue with subsequent variant calling was conducted as outlined by the Broad Institute's best practices and used to identify somatic mutations within each tumor.

Results: We have identified 28,935 unique MHC class I peptides from 2285 known proteins from six different PDAC donors, with peptides from a further three PDAC donors isolated and awaiting mass spectrometry analysis. Among these peptides, there are ***285 unique peptides sequences bearing one or more missense substitutions identified from 106 mutated proteins.*** The presence of these mutations identified through mass spectrometry was confirmed using the identified panel of somatic mutations generated from the WES of tumor tissue. *In silico* analysis confirms that isolated peptides have binding affinities specific for the MHC alleles of each given PDAC donor. Immunogenicity score modeling to assess for ability to elicit CD8+ T-cell reactive responses indicates that our isolated mutated peptide sequences are predicted to be more immunogenic than non-mutated sequences. Interestingly, we have uncovered a population of peptide sequences conserved across different PDAC donors that share specfic MHC alleles, implicating potential neoantigen translatability across patients bearing overlapping MHC alleles.

Conclusions: We have been able to identify peptide sequences with the same characteristics of MHC class I presented peptides directly from PDAC tumors. Analysis of these peptides indicates conservation of specific peptide sequence presentation across the same MHC class I allele in different patients. 1% of identified peptide sequences contain known mutations independently verified to be present within the tumor through WES. *In silico* predictions call these mutated sequences as significantly immunogenic, hinting at their potential to be clinically-significant neoantigens. This method unlocks new avenues for identifying clinically relevant neoantigens that are translatable across patients bearing overapping MHC alleles.

Title: LONG-TERM SURVIVAL RATES OF BURKITT LYMPHOMA PATIENTS WITH HIV – A NATIONAL CANCER DATABASE (NCDB) STUDY

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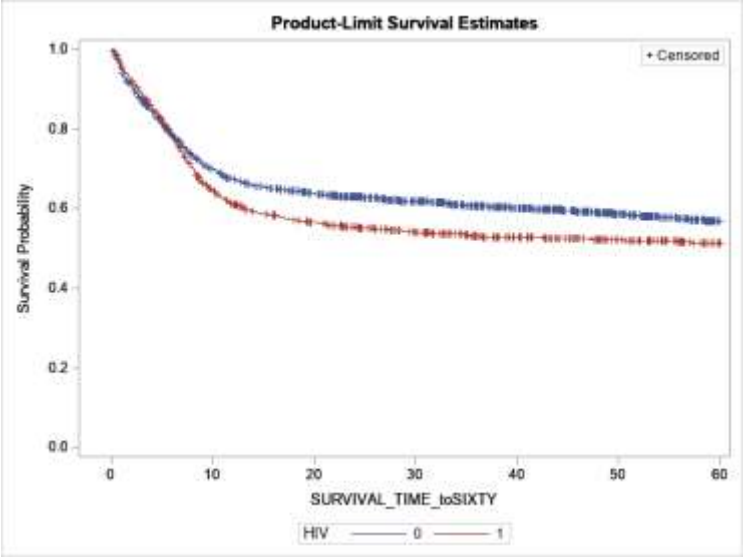
Background and Significance: Burkitt lymphoma (BL) accounts for 10–35% of AIDS-defining lymphoma in people with HIV. It is the second most common subtype of non-Hodgkin's lymphoma that occurs in HIV-positive patients with a relatively high CD4 cell count. Previous research consisting of cohorts of less than 300 patients have shown decreased survival for HIV-associated BL. This study aims to compare Burkitt lymphoma mortality in individuals with and without HIV, while also investigating additional predictors of mortality in HIV-associated Burkitt lymphoma.

Methods: Using the 2004-2019 NCDB, we identified 4,312 patients with stage 3 or 4 Burkitt lymphoma, had a known HIV status, and received either chemotherapy alone or chemotherapy and immunotherapy. Time-to-death was evaluated using Kaplan-Meier survival estimates. Risk of death was evaluated using an extended multivariable Cox model adjusted for multiple factors (table 1) and with a Heaviside function for HIV status by time period (0-3 month vs 3-60 month).

Results/Data: Of the 4,312 patients included, the majority were white (80%), male (77%), and non-Hispanic (87%), and 1,514 (35%) had HIV. For months 0-3 from time of diagnosis, HIV status was not associated with a statistically significant increase in risk of death (HR = 1.04, 95% CI: 0.86, 1.26, p = .6648). From month 3-60 HIV status was associated with a 55% increase in risk of death (95% CI: 1.38, 1.75, p <.0001). Further, this difference in hazard rates (0-3 vs 3-60) was statistically significant (HR = 1.49, 95% CI: 1.22 – 1.82, p <.001).

Conclusions: There is an increased mortality rate from months 3-60 in BL patients with HIV compared to patients without HIV, supporting previously published literature. Additionally, risk of death is significantly decreased in patients treated with combination chemotherapy and immunotherapy compared to chemotherapy alone, providing valuable clinical insight into treatment decision making in the care of HIV-associated BL.



Parameter	Hazard Ratio	95% CI		*P*
HIV vs NO HIV 0 – 3 Months	1.04	0.86	1.26	.6668
HIV vs NO HIV 3 – 60 Months	1.55	1.38	1.75	<.0001
Chemo and Immuno vs Chemo Only	0.59	0.53	0.65	<.0001
Extranodal vs Nodal	0.93	0.83	1.04	.2083
Age (1 year change)	1.03	1.02	1.03	<.0001
Female vs Male	0.86	0.77	0.96	.0090
Black vs White	1.11	0.97	1.27	.1300
Other vs White	1.00	0.81	1.23	.9922
Hispanic vs Not Hispanic	0.84	0.73	0.98	.0231