THE ROLE OF LIGANDS OF ACTIVATORY RECEPTOR NKGD2 IN THE IMMUNE-DEPENDENT PATHOGENESIS AND EVOLUTION OF INFLAMMATORY BOWEL DISEASE (IBD)

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Background Long-term inflammation in IBD is mediated by several immune cells, including T lymphocytes and natural killer (NK) cells, through the engagement of NK group 2D (NKGD2) receptors. Allelic variations of NKGD2 ligands (NKGD2Ls, MICA/B, ULBP1-3) influence differential levels and localization of protein expression or the release of soluble isofoms. The affinity of interaction with NKGD2 can be also affected, modulating the cytotoxic activity of the target cell. Evaluation of these molecular pathways and soluble ligands presents the potential utility of a clinical biomarker for patient outcomes.

Methods Gut tissue biopsies (left and right sides) and peripheral blood were collected from patients. 10 pediatric and 11 adult patients with IBD, 10 patients with bowel malignancies and history of IBD were included in the study. Plasma form IBD patients and 10 healthy donors as controls, was used to quantify soluble NKGD2Ls (sNKGD2Ls) by ELISA (R&D Systems Duo Set). Nucleic acids were extracted from gut biopsies using the BioMasher II (Kimble) and All Prep DNA/RNA universal kit (Qiagen). Single nucleotide polymorphisms (SNPs, N=26) and relative gene expression of NKGD2L genes were conducted by qPCR using Taqman assays.

Results 9/11 adult patients had diagnosis of ulcerative colitis, compared to 3/10 pediatric. 5/10 pediatrics had Crohn’s disease and 2/10 unclassified IBD. A trend of prevalence of some allelic variants was detected for most of NKGD2Ls. In addition, mRNA encoding for NKGD2Ls was detected commonly, although with heterogeneous quantifications, in all the tissues, including the retrospectively collected malignancies with history of IBD. Interestingly, the levels of sNKGD2Ls were higher in pediatric (p<0.001) as compared to adult patients. No or low levels of sNKGD2Ls were detectable in healthy donors. Moreover 3/5 patients with the highest level (700–1500 pg/ml) of sMICA had homozygosity at least in one of the rs1051792 or rs1051794 polymorphic site (GG allele variant MICA-129Val or MICA-250Val) that have been reported to be associated with soluble form of MICA.

Conclusions These results, although preliminary and further investigations are ongoing, suggest the relevance of NKGD2/NKGD2L pathway in the development and evolution of IBD. sNKGD2Ls could be detected in most of patients, with different levels and highest concentrations in pediatric patients. In some cases, the presence of sNKGD2Ls in the plasma could be associated with defined polymorphisms in genes encoding for these proteins.
pre-treatment and on-treatment biopsies demonstrated intratumoral suppression of KYN production with EPA 600 mg BID.

Conclusions Using suppression of plasma KYN as a pharmacodynamic marker of EPA activity, we demonstrated that maximal blockade of IDO1 activity in the context of anti-PD-1 treatment requires doses of EPA substantially higher than those tested in prior clinical studies. These findings are now informing additional proof of concept clinical studies.

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Ethics Approval These studies were each approved by the institutional review board or independent ethics committee of participating institutions.

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PROFILING TUMOR CIRCULATING CELL-FREE DNA WITH AN ENHANCED WHOLE-EXOME TO ENABLE SENSITIVE ASSESSMENT OF SOMATIC MUTATIONS

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Background An increasing number of studies have demonstrated the potential use of circulating cell-free DNA (cfDNA) for diagnosis, prognosis, disease progression, and treatment monitoring. However, many of these studies use assays covering a limited set of genes and therefore miss biologically and clinically relevant genetic alterations involving immuno-modulatory pathways which confer treatment resistance, and leading to changes in neoantigen status. To address this, we developed a whole-exome scale cfDNA platform, NeXT Liquid Biopsy™, that enables sensitive detection and tracking of mutations in approximately 20000 genes.

Methods To enable sensitive detection across the exome, our enhanced exome assay and chemistry augments hard-to-sequence genomic regions, such as regions of high GC content, to enable more uniform coverage across the exome. We achieved a high mean sequencing depth of approximately 2000X exome-wide, with additionally boosted depth for 248 clinically relevant oncogenic and tumor suppressor genes to further enhance sensitivity. We developed a computational pipeline for our NeXT Liquid Biopsy assay optimized to lower the noise floor for variant detection, providing sensitive monitoring and de novo detection of variants over multiple time points.

Results We evaluated the sensitivity of our NeXT Liquid Biopsy platform in three ways. First, we evaluated the sensitivity within the coverage boosted regions using the SeraCare reference materials at multiple allele frequency (AF) dilutions. Our platform identified all 8 and 25 Horizon and SeraCare SNV events at 1% AF and above, respectively, and detected 24 out of 25 events at 0.5% for the SeraCare samples. Additionally, to enable sensitivity analysis at the whole-exome scale, we then developed a cell culture media system that models the shedding of tumor DNA fragments seen in human plasma samples and created tumor/normal dilution series in vitro. We achieved >95% sensitivity for variants with AF≥2%, and between 85% to 92% for mutations with AF of 1%-2%. Second, we evaluated false-positive rates on 12 cancer patients using digital droplet PCR. Third, we demonstrated our ability to longitudinally monitor treatment response using a clinical cancer cohort on checkpoint therapy, profiling putative tumor evolution while on therapy.

Conclusions In conclusion, we have developed a whole-exome scale liquid biopsy platform, NeXT Liquid Biopsy, that enables sensitive monitoring and detection of somatic SNVs from cfDNA across ~20000 genes. The platform enables broader monitoring of changes in response to cancer therapy, acquired mechanisms of resistance, and intra- and inter-tumor heterogeneity.

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NLR (NEUTROPHIL LYMPHOCYTE RATIO) AND PLR (PLATELET LYMPHOCYTE RATIO) CHANGES AS A PREDICTOR OF EVENTUAL TREATMENT FAILURE AND DEATH ON NIVOLUMAB THERAPY IN RENAL CELL CARCINOMA

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Background Elevated baseline neutrophil lymphocyte ratio ratios (NLR) are now well established as a poor predictor of survival in renal cell carcinoma (RCC) and other cancers. Platelet Lymphocyte Ratios (PLR) have also recently shown similar effects. Despite these findings, the practical use of these ratios is still somewhat limited. We have previously shown that higher NLRs may be associated with increased concentrations of myeloid derived suppressor cells (MDSC). We hypothesized that increases in NLR or PLR (NLR/PLR failure) at 2 months while on immunotherapy could be a predictor of eventual treatment failure and overall survival.

Methods We analyzed patients who received nivolumab therapy for RCC at our institution from 3/2016 to 6/2019. Patients with complete data on NLR and PLR at time = 0 and +2 months and those who had accurate response and overall survival information available were selected (n = 37). Information on comorbidities, previous therapy, demographics were collected for adjusted analysis. NLR failure was defined as an increase of 3 or more compared to baseline NLR. Cox proportional hazard models were used to analyze the risk of progression and death with NLR/PLR failure at 2 months (± 2 weeks). Kaplan Meier graphs were constructed to trace survival functions for PFS and OS by NLR.

Results NLR failure was associated with a statistically significant increase in the risk of progression on nivolumab therapy (HR 4.26, 95% CI [1.47–12.3], p = 0.007), in an adjusted cox regression model that included baseline NLR. In this adjusted model, the value of baseline NLR to predict