FINAL REPORT

Developing an RNA-based Strategy for Actionable Target Assessment to Personalize Cancer Therapies

Background

There is increasing interest in personalized cancer therapy. Although the first wave of precision oncology has focused on genomically informed therapy, many tumors do not have a single strong genomic driver, or have concurrent alterations that may confer resistance. Thus, the "single gene – single targeted therapy" approach is effective only for a limited number of patients.

New-generation antibody-drug conjugates (ADCs) have shown significant promise. However, ADC trials often screen for one ADC target at a time, leading to inefficiencies in patient identification and delays in patient treatment. There is a great need for **a multiplex test that can rapidly test for multiple ADC targets** — targets that cannot be identified through traditional DNA-sequencing approaches.

Since ADCs target proteins on the cell surface, it is most appropriate to assess potential targets by their overexpression at the RNA level. RNA overexpression is likely to select for patients who have protein overexpression, so we believe an RNA-based multiplex diagnostic test would be highly beneficial in matching patients to therapies. Furthermore, a multiplex test could support histology-agnostic trials, facilitating signal seeking across diseases, including rare tumor types.

There is strong rationale for screening for overexpression of ADC targets using an RNA-based diagnostic. Although ADCs target cell-surface proteins, many ADC targets were identified by their overexpression at the RNA level. RNA levels have been found to strongly correlate with some ADC targets already. Thus, we propose to establish an RNA-based multiplex diagnostic assay to facilitate screening for multiple therapeutic targets and identifying patients with RNA and protein overexpression involving **targets of interest (TOIs)**. These TOIs could be targets of ADCs (such as HER2 or mesothelin) or potential biomarkers for small molecule inhibitors (*e.g.*, fibroblast growth factor receptors).

In this study, we proposed to use the nCounter platform from NanoString Technologies, a well-established, robust method to assess messenger RNA (mRNA) expression in formalin-fixed paraffin-embedded (FFPE) samples. The nCounter Analysis System uses novel digital barcode technology and single-molecule imaging for direct hybridization and detection of hundreds of unique transcripts in a single reaction; this has high levels of precision and sensitivity (<1 copy per cell). This platform has the advantage that mRNA does not need to be converted to complementary DNA (cDNA) by reverse transcription and there is no need to amplify cDNA.

Objectives and Results

Our long-term goal is **to optimize personalized cancer therapy through selection of optimal targets** identified using more comprehensive methods than DNA sequencing alone. Our short-term goal is **to develop a multiplex assay for ADC targets** that can be used to identify patients for immunohistochemistry (IHC)-based screening for ADC trials. We hypothesize that by multiplex RNA testing, we can optimally match ADCs or bispecific antibodies to personalize therapy. We expect that our RNA multiplex diagnostic will be implemented to initiate novel (and support existing) trials using these therapeutic options. We pursued our goals through two Specific Aims:

Specific Aim 1. To establish an integrated RNA-based multiplex assay in the pre-CLIA (Clinical Laboratory Improvement Amendments) environment.

In collaboration with NanoString, we have already designed a multiplex RNA assay with probes selected for many of the ADC targets in the public domain, including agents in the cancer therapy evaluation program (CTEP) portfolio. We thus established this assay in the pre-CLIA environment and established its preliminary analytic validity, including intra- and inter-assay reproducibility and inter-operator reproducibility.

We have next expanded the assay to increase its scope, and have initiated Phase 0 validation in the CLIA Clinical Translational Laboratory Unit. This work is still ongoing, but has already shown (**a**) correlation between protein expression by western blotting and RNA expression by TARGET assay, and (**b**) evidence that cell lines highly expressing TROP2 are usually sensitive to ADCs. Figure 1 demonstrates TROP2 expression and efficacy of treatment with the ADC sacituzumab govitecan and its unconjugated payload SN-38 in multiple cell lines.

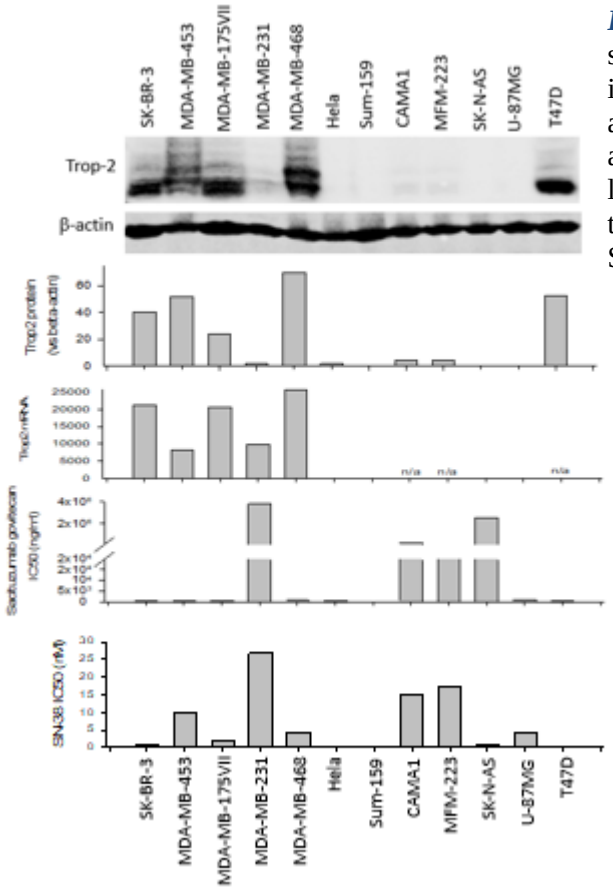

Figure 1. TROP2 expression and efficacy of treatment with sacituzumab govitecan and its unconjugated payload SN-38 in multiple cell lines. TROP2 and actin expression was assessed on cell line lysates by western blotting (top 2 rows) and TROP2 RNA expression was assessed in the same cell lines by TARGET assay (third row). Cell lines were then treated with the TROP2 ADC sacituzumab govitecan or SN38, and assayed for viability (bottom two rows).

Specific Aim 2. To compare target expression using our multiplex assay and IHC.

We had proposed to assess the expression of selected targets using both our multiplex assay and the IHC approaches currently used for patient selection. We first established IHC protocols for FFPE tissues. For selected biomarkers, such as HER2, IHC staining was already established in the CLIA laboratory in the Department of Pathology. For this project, we established additional IHC assays in the pre-CLIA laboratory and transitioned them to the CLIA laboratory in preparation of finalizing CLIA validation of the TARGET assay. As shown in Figure 2, IHC in the lab demonstrated that IHC can be used to show differential expression of TROP2 in cell lines, correlating with findings on western blotting. Further, analysis of patient samples by TARGET assay and IHC demonstrated that high TROP2 RNA expression on TARGET was associated with high TROP2 protein expression on IHC.

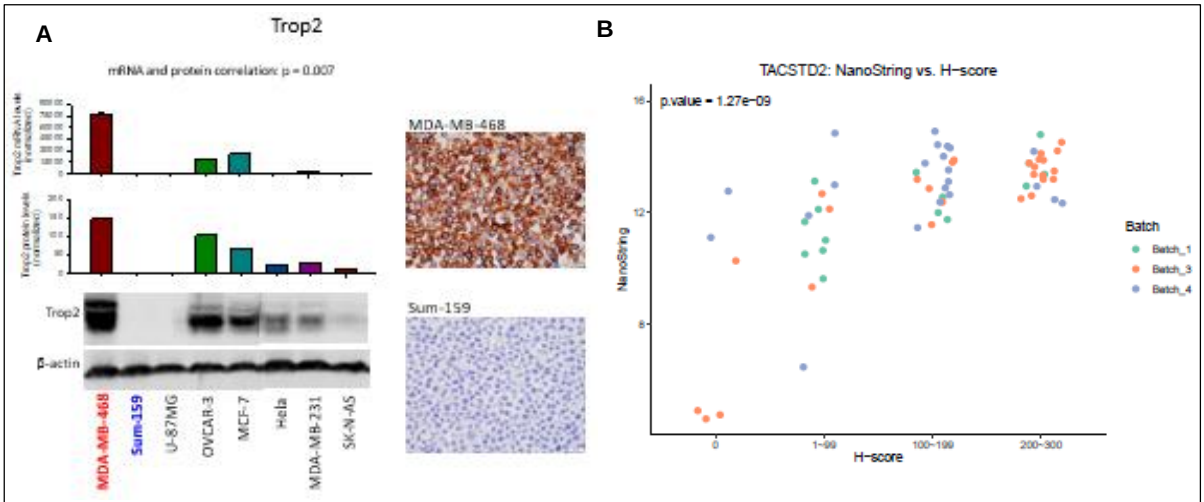

Figure 2. TACTD2 RNA expression by TARGET vs TROP2 protein expression by IHC. analysis. MDA-MB-468 and SUM159 were used as positive and negative controls for IHC.

Future Directions

As originally proposed, we have developed a multiplex assay to rapidly screen for ADC targets. A full manuscript describing this work is currently being prepared for publication while the assay is undergoing CLIA validation in the Clinical Translational Laboratory Unit to serve as a central lab for trials using this assay for patient enrollment. Implementation of multiplex screening will allow us to test for expression of targets, such as TROP2 or HER2, which are not regularly tested in many tumor types. Importantly, we have already gotten preliminary approval to use this assay in an upcoming investigator-initiated trial ADC-MATCH that will be launched as a multicenter CTEP trial. We expect to validate additional targets over the next year through other funding sources in order to further enhance the clinical utility of testing the actionable transcriptome.