#3706/19 Validation of human and mouse myeloid panels on the NanoString® nCounter® Platform

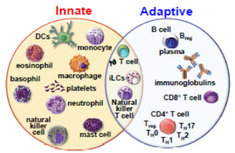
Sushil Kumar[1], Sarah Warren[2], Tiziana Cotechini[1], Terry Medler[1], Wenjie Xu[2] , Erin Piazza[2], Patrick Flynn[1], Christina Bailey[2], Joseph M. Beechem[2], Lisa M. Coussens[1].
[1]Oregon Health and Science University, Portland, OR; [2]NanoString Technologies, Seattle, WA

NanoString Technologies
530 Fairview Ave North Seattle, WA 98109

Abstract

The innate immune system profoundly influences cancer development as well as response to various therapeutic approaches. Multiple myeloid cell lineages are central to the innate immune response during cancer and have been found to regulate diverse cellular processes encompassing immune suppression, angiogenesis, invasion, metastasis, and drug resistance.

In collaboration with Dr. Lisa Coussens and scientists at Oregon Health and Science University (OHSU), NanoString has created two multi-plexed gene expression panels for human and mouse studies to profile the myeloid component of the innate immune response influencing immuno-regulation.



Kumar, Chan, & Coussens (2016) Encyclopedia of Immunobiology

The panels provide a targeted gene expression assay to both quantitatively and qualitatively measure attributes of the innate immune response by evaluating relative enrichment of genes reflecting recruitment, differentiation, presence and effector phenotype of select myeloid subtypes (e.g., granulocytes, dendritic cells, monocytes, macrophages, mast cells), and thus their relative influence on the adaptive immune components in tissues. This assay therefore enables qualitative assessment of tissue microenvironments towards immunoregulation, and in the future also can serve as a discovery platform for biomarker identification and prognostic signature development for novel immunotherapeutics.

Methods

Gene content for human (692 genes) and murine versions (675 genes) of the myeloid panels were developed from in-house studies and data mining from publicly available datasets. The aim of this study was to validate panel content by profiling whole tumors, as well as sorted tumor-associated macrophages (TAMs), in syngeneic murine models of mesothelioma and mammary tumorigenesis. The gene expression data are compared to flow cytometry analysis of myeloid components in the tumor microenvironment. The same tumors are also profiled with the NanoString PanCancer Immune Profiling panel to further evaluate adaptive immune responses.

Sample Input Optimization

Direct Hybridization Protocol Enables Profiling of ≥ 5 ng RNA

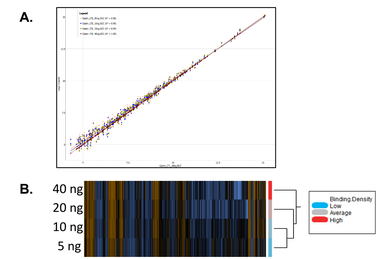


Fig. 1: Total RNA was extracted from sorted TAMs and hybridized with the NanoString myeloid panel at varying concentrations (5 ng, 10ng, 20ng, 40 ng). Despite low binding density, gene expression patterns were consistent across input levels, as shown in the scatter plot (**A**) and the heat map (**B**).

Low Input Protocol Enables Profiling of ≥ 0.5 ng RNA

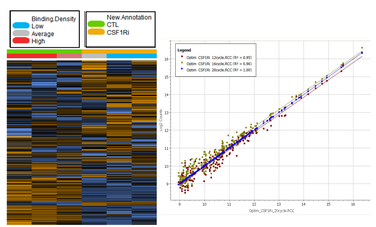


Fig. 2: Total RNA (0.75 ng/sample) was converted into cDNA and pre-amplification was performed using primer pools specific for genes in the myeloid panel. 12-20 cycles of pre-amplification were utilized for gene enrichment analysis. (**A**) Heat map representation of transcriptional changes reveals gene enrichment comparing sorted TAMs from vehicle control treated tumor. Although 20 cycles were required for quantitative assessment of lower RNA yielding tumors, as in the case of CSF1Ri-treated tumors where a majority of TAMs were depleted, gene expression was observable even in samples with 12 pre-amplification rounds (**B**).

Myeloid Panel Reveals Differences in Myeloid Gene Expression Profiles of Distinct Tumor Types

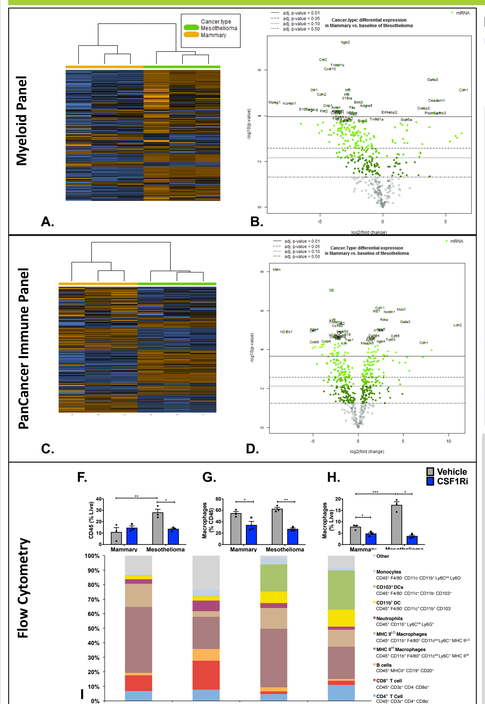


Fig. 3: Whole tumor samples were utilized for RNA extraction and direct hybridization with myeloid panel probesets or PanCancer Immune panel probesets. Analysis by heatmaps (**A, C, E**) or volcano plots (**B** and **D**) reveal distinct immune microenvironments in mesothelioma as compared to mammary carcinomas. Assessment of these using the myeloid panel reveals significantly enriched myeloid gene expression that reflects TAM abundance in these tumors as assessed by flow cytometry (**F, G, H & I**).

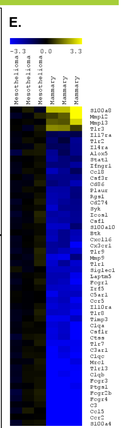
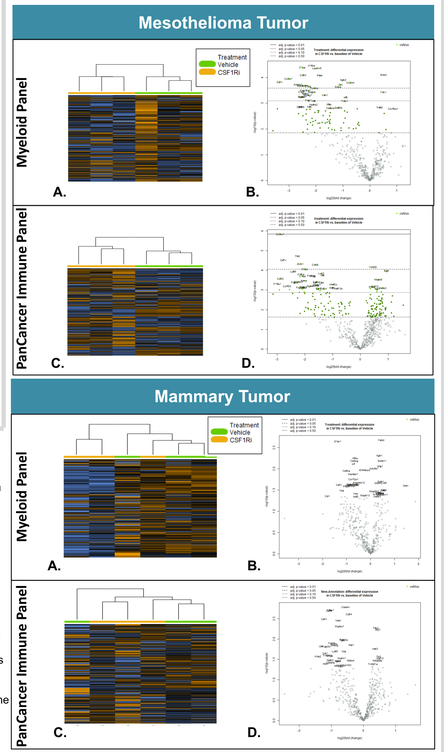


Fig. 4: Whole tumor samples of murine mesothelioma and mammary carcinoma were compared following in vivo TAM depletion via colony stimulating factor-1 receptor (CSF-1R) inhibitor administration. TAM depletion was validated by flow cytometry (**Fig. 3 E-H**). Gene expression assessment from TAM-depleted tumors reveals significant changes in gene expression by both the myeloid and PanCancer Immune Panel (**A-D**).

CSF1R Blockade in the Tumor Microenvironment Confers Differential Gene Expression Patterns



Gene Expression Profiling of Sorted TAMs

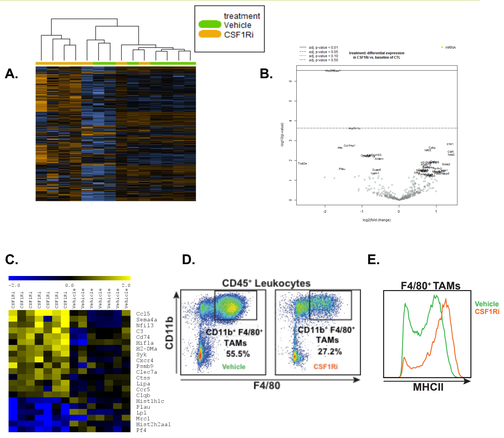


Fig. 5 To quantitatively evaluate TAM reprogramming by gene expression changes using the myeloid panel, flow sorted TAMs from CSF1Ri vs. vehicle-treated tumors were utilized. Samples were subjected to 16 cycles of pre-amplification to achieve optimal gene enrichment prior to hybridization with the myeloid panel probeset and reveals modulation in overall TAM gene expression due to CSF-1R blockade (**A-C**) Moreover, the most significant gene expression changes in TAMs following CSF-1R inhibition were reflective of transcriptional reprogramming favoring a T_H1-type phenotype and accompanying altered TAM phenotype as indicated by FACS analysis of tumors (**D**), and altered MHCII expression (**E**).

Conclusions

- The myeloid gene panel enables robust examination of myeloid cell presence in tissues/tumors and transcriptional assessment of their phenotype.
- The TAM-specific gene content of the panel enables reduced RNA input requirements thus supporting quantitative assessment of sorted TAMs or small tumors where RNA may be limiting.
- The myeloid panel performed well in analyzing transcriptional reprogramming following therapeutic targeting to reveal T_H1 vs T_H2 propensity and is therefore suitable for predicting immunogenic vs immunosuppressive features of tumor microenvironments.