**Supplementary Online-Only Material**

**Supplementary Methods**

Tumor tissue biopsies were obtained from 19 patients from the dose expansion cohort at screening (pretreatment) and after treatment at cycle 1 (post-treatment; after 3 doses of MEDI-573). The majority of biopsy samples were collected from multiple metastatic sites including lymph node, lung, liver, and bone. Eleven of 19 (58%) patients had usable paired pretreatment and post-treatment mRNA samples. Samples collected by fine needle aspiration (into 2 mL cryovial tubes with 0.4 mL miVana miRNA lysis buffer) and those collected by standard biopsy were rapidly frozen immediately after collection and were stored at -70°C or below and shipped on dry ice to MedImmune for analysis.

The standard tumor biopsy samples contained a mixture of normal tissue and tumor tissue. To eliminate the normal tissue and enrich the sample for the tumor tissue, the samples were embedded in optimal cutting temperature compound (OCT) and dissected with laser-capture microdissection (LCM) upon receipt at MedImmune. mRNA was extracted from all samples by using ZR RNA MicroPrep Kit (Zymo Research, Irvine, CA).

Single-stranded cDNA was generated from total RNA using the SuperScript® III First-Strand Synthesis SuperMix, and samples of cDNA were pre-amplified using TaqMan Pre-Amp Master Mix. Reactions contained 5 μL of cDNA, 10 μL of Pre-Amp Master Mix, and 5 μL of 0.2 × gene expression assay mix (comprising all primer/probes to be assayed) at a final reaction volume of 20 μL. PCR reactions were cycled with the recommended 14-cycle and then diluted 1:5 with TE buffer. Pre-amplified cDNA was used immediately or stored at −20°C until processed. The reaction mix for preparing samples was loaded into 48 × 48 dynamic array chips and contained 2.5 μL of 2× Universal Master Mix, 0.25 μL of Sample Loading Buffer, and 2.25 μL of preamplified cDNA. The reaction mix for primer/probes contained 2.5 μL of 20 ×TaqMan Gene Expression Assay and 2.5 μL of Assay Loading Buffer. Prior to loading the samples and assay reagents into the inlets, the chip was primed in the IFC (Fluidic Circuit Controller) Controller. Samples (5 μL) were loaded into each sample inlet of the dynamic array chip, and 5 μL of 10× Gene Expression Assay Mix was loaded into each detector inlet. The chip was placed on the IFC Controller for loading and mixing. Upon completion of the IFC priming step, the chip was loaded on the BioMark RT-PCR System for thermal cycling (95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, 60°C for one minute). The number of replicates and the composition of the samples varied depending on the particular experiment but were never less than triplicate determinations. Average cycle threshold (Ct) values were used to quantify of the designed Taqman assays. The average Ct values of all available reference gene assays within a sample were utilized for calculation of ΔCt (ΔCT = the average CT of target gene – the average CT of the reference gene.)

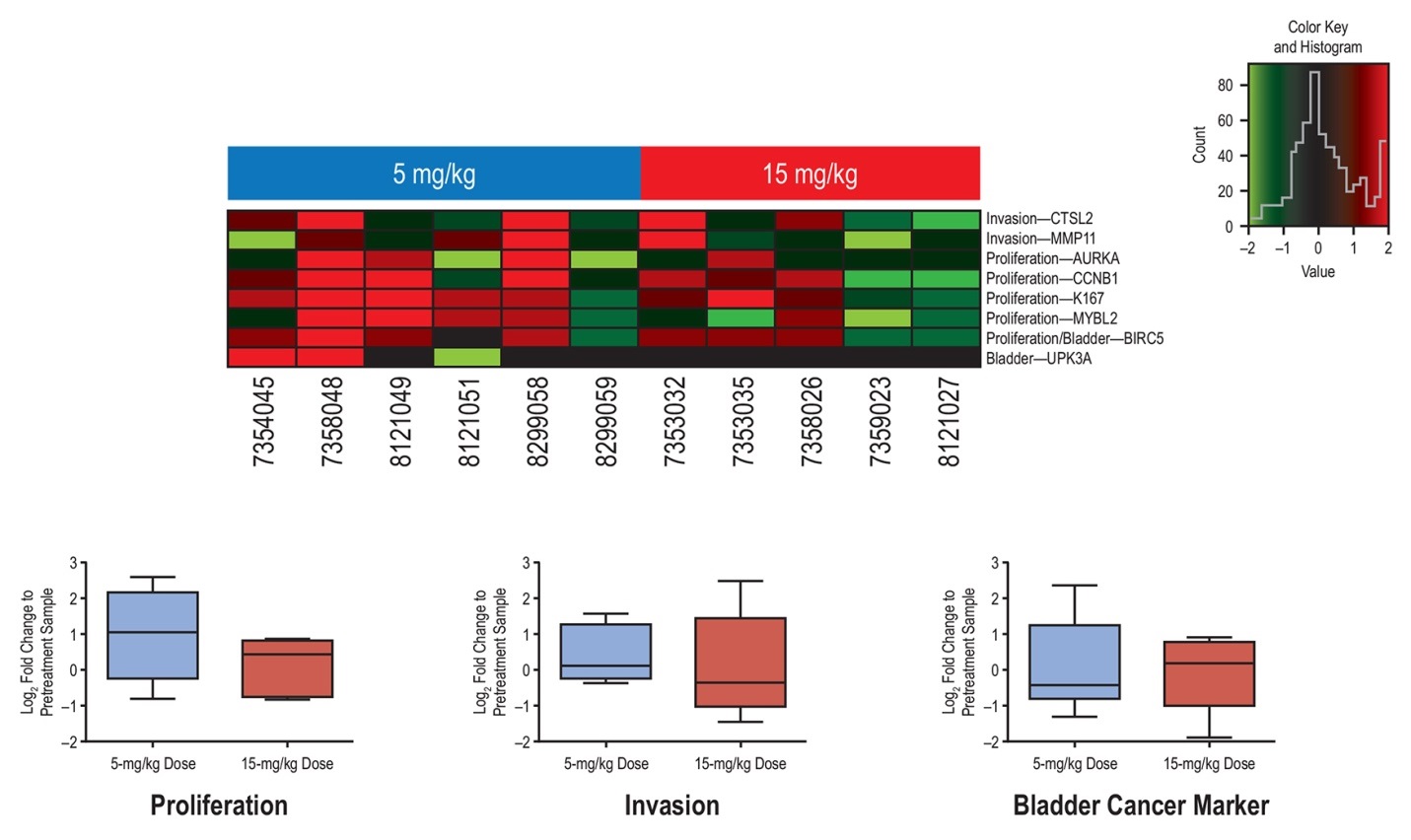
The mRNA expression levels of *IGF1, IGF2, IGF1R* and IR-A, individual proliferation genes (*AURKA, CCNB1, MKI67,* and *MYBL2*) and cancer invasion of genes (*CTSL2* and *MMP11*) and the bladder cancer maker, UPK3A, in tumor biopsy samples were measured as Ct and samples were normalized to the average expression levels of the two housekeeping genes beta-actin (ACTB) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The normalized expression values are represented by ΔCt. The differential expression level between post-treatment tumor biopsy samples and pretreatment samples was determined by calculating the fold changes for genes of interest using the formula 2-ΔΔCt, where ΔΔCt for the gene of interest is defined as: (mean ΔCt of pretreatment samples – ΔCt of the post-treatment sample) and the relative expression level of the gene of interest was represented by −ΔCt wherein −ΔCt = - [(mean Ct of 2 housekeeping genes) – (Ct gene of interest)]. The invasion composite score is calculated as the mean fold changes of the genes *MMP11* and *CTSL2*, while the proliferation composite score is the mean fold changes of the genes *KI67, AURKA, BIRC5, CCNB1* and *MYBL2*.

**Supplementary Figure Legend**

**Figure S1.** Top: mRNA differential expression of individual proliferation genes (*AURKA, CCNB1, MKI67,* and *MYBL2*) and cancer invasion genes (*CTSL2* and *MMP11*) and the bladder cancer marker, UPK3A, in biopsy samples (from patients with bladder cancer at pretreatment and after treatment at 5 mg/kg and 15 mg/kg. Bottom: Composite differential expression score from pretreatment and post-treatment tissues

of proliferation, invasion, and bladder cancer markers in cohorts 5 mg/kg and 15 mg/kg.

**Figure S1.**

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