PRO140 CCR5

CONFIDENTIAL

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1. Report Summary

- Sensitivity screening for labeled leronlimab (PRO140 CCR5 Hu IgG4a) utilized a panel of 63 evaluable samples from 9 cancer indications as follows: 7 Non-Small Cell Lung Carcinoma (NSCLC) Adenocarcinoma (Lung Adeno), 9 NSCLC Squamous Cell Carcinoma (Lung SCC), 5 Triple Negative Breast Cancer (TNBC), 5 Breast Cancer (non-TNBC), 7 Pancreatic Cancer, 6 Urothelial Cancer, 9 Colorectal Cancer, 7 Head and Neck (H&N) Cancer, and 8 Sarcoma.

- CCR5 reactivity in tumor cells was evaluated using Percent Scores (sum of percentages at intensities ≥1+, ≥2+, and ≥3+ with values ranging from 0 to 100). Using this approach, the values for Percent Scores ≥1+ are equivalent to the overall percentage of tumor cells (0-100%) with CCR5 staining (% Positive Tumor Cells). H-Scores were also calculated [sum of each percentage (0-100%) multiplied by its corresponding intensity (0, 1+, 2+, 3+) with scores ranging from 0-300].

- Across the entire sample set, CCR5 displayed a full range of reactivity with H-Scores observed from 0–300. However overall, most of the 63 cases evaluated tended to have either H-Scores >175 (highly reactive) (24/63) or <100 (low or non-reactive) (32/63). Very few samples (7/63) fell into a middle range with H-Scores from 100–175. Approximately half of the samples with H-Scores <100 lacked CCR5 expression (H-Score = 0).

- Within each cancer indication, the following Average H-Scores were observed- Urothelial Cancer: 183.3, Breast Cancer: 164.0, Lung SCC: 162.8, Colorectal Cancer: 136.0, Lung Adeno: 125.1, Pancreatic Cancer: 112.9, H&N Cancer: 68.4, TNBC: 44.0, and Sarcoma: 23.6.

- To analyze CCR5 reactivity in the screening samples, a ≥ 1 + staining threshold was used. For example, if using ≥1+ staining intensity in ≥50% of tumor cells (or % Positive Tumor Cells≥50) as a cut-off for CCR5 positivity, the following percentage of cases per indication would be considered positive- Lung Adeno: 57%, Lung SCC: 78%, TNBC: 20%, Breast Cancer: 80%, Pancreatic Cancer: 57%, Urothelial Cancer: 67%, Colorectal Cancer: 78%, H&N Cancer: 29%, and Sarcoma: 13%.

- CCR5 precision and reproducibility testing (assay validation) was performed using a subset of samples from the sensitivity screen. Successful validation was achieved through robust variability testing for intra-assay (precision) and inter-assay (reproducibility) in 4 samples that represented a range of CCR5 expression. Acceptance criteria were met for the CCR5 (Hu IgG4a) IHC assay with a standard deviation (Std Dev) that did not exceed 6.8, a standard error of the mean (SEM) that did not exceed 2.8, and a coefficient of variation (CV) did not exceed 8% for all sample sets.

2. Scoring Scheme

All pathology analysis and scoring for CCR5 (Hu IgG4) is performed by a board-certified pathologist. Evaluation is semi-quantitative with numeric scoring data provided as a measure of reactivity. The guidelines used for scoring CCR5 as detected by IHC in FFPE tumor samples are as follows:

- H&E (hematoxylin and eosin)-stained slides of all specimens are evaluated for the presence of tumor cells.
- CCR5 is reactive in the cytoplasm of a subset of tumor cells. CCR5 cytoplasmic staining can be observed throughout the cytoplasm, associated with the plasma membrane, beneath the plasma membrane (submembranous), at the nuclear membrane (diffuse or fine granular), and facing the lumen of tubules (adluminal). All localizations are evaluated together. If present, true membrane staining cannot be distinguished from membrane-associated staining (the signal density obscures separation) and therefore is included in the cytoplasmic score.
- Only tumor cells are scored for CCR5. That is, scoring excludes any surrounding staining in stroma, areas of non-tumor, and adjacent normal tissue. Ischemic or necrotic areas are not scored in any indication.
- The main components to scoring for CCR5 include percentages at differential intensities to determine Percent Scores and H-Scores (described below). Using this method, Percent Scores ≥1+ are equivalent to the overall percentage of tumor cells (0-100%) with CCR5 staining (% Positive Tumor Cells).
- Percent Scores and H-Scores both require recording the percentage of cells with CCR5 staining at a corresponding differential intensity on a four-point semi-quantitative scale (0, 1+, 2+, 3+).
- On the scoring scale: 0= null, negative or non-specific staining, 1+= low or weak staining, 2+= medium or moderate staining, and 3+= high or strong staining.
- The percentage of cells staining at each intensity is estimated directly and typically reported as one of the following; though other increments can also be used at the pathologist's discretion: 0, 1, 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, 99, or 100%.

Percent Score Method

Percent Scores are calculated by summing the percentages of intensities at either ≥1+, ≥2+ or ≥3+. Thus, scores range from 0 to 100.

Percent Score ≥1+ = (% at 1+**) + (**% at 2+**) + (**% at 3+**)**
Percent Score ≥2+ = (% at 2+**) + (**% at 3+**)**
Percent Score ≥3+ = (% at 3+**)**

H-Score Method

The H-Score is calculated by summing the percentage of cells with intensity of expression (brown staining) multiplied by their corresponding differential intensity on a four-point semi-quantitative scale (0, 1+, 2+, 3+). Thus, scores range from 0 to 300.

H-Score = [(% at <1) x 0**] + [** (% at 1+) x 1**] + [** (% at 2+) x 2**] + [** (% at 3+) x 3**]**

Table 1. Sensitivity Screen for CCR5 (Hu IgG4a) in Human Cancer Tissues

Sensitivity Screen for CCR5 Human IgG4a in Various Tumors													
Sample Information				CCR5 Cytoplasmic Tumor Staining								Additional Sample Information	Comments
				% at Differential Intensities				%	%	%			
Slide #	Tissue Type	Q/MTB#	Sample ID	0	1+	2+	3+	Staining ≥1+	Staining ≥2+	Staining ≥3+	H-Score	Pathology	
2	**Lung Adeno**	QMTB396-1	Q05043-01	70	24	1	5	30	6	5	41	Lung Adenocarcinoma. Non-Small Cell Lung Carcinoma (NSCLC).	Poorly differentiated.
2	**Lung Adeno**	QMTB396-2	Q05040-01	5	70	5	20	95	25	20	140	Lung Adenocarcinoma (NSCLC) with micropapillary features.	Poorly to moderately differentiated.
2	**Lung Adeno**	QMTB396-3	Q01951-01	10	20	40	30	90	70	30	190	Lung Adenocarcinoma. Non-Small Cell Lung Carcinoma (NSCLC).	Well differentiated. Includes Membranous/submembranous CCR5 signa.
2	**Lung Adeno**	QMTB396-4	Q09362-03	0	10	20	70	100	90	70	260	Lung Adenocarcinoma. Non-Small Cell Lung Carcinoma (NSCLC).	-
2	**Lung Adeno**	QMTB396-5	Q09347-02	5	50	5	40	95	45	40	180	Lung Adenocarcinoma. Non-Small Cell Lung Carcinoma (NSCLC).	Some fixation artifact.
2	***Lung Adeno***	*QMTB396-6*	*Q09349-01*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*Lung Adenocarcinoma (NSCLC) with prominent lymphoplasmacytic infiltrate.*	*Significant fixation artifact. Sample could not be scored.*
2	**Lung Adeno**	QMTB396-7	Q09351-02	85	5	5	5	15	10	5	30	Appears as Lung Squamous Cell Carcinoma and not Adenocarcinoma.	-
2	**Lung Adeno**	QMTB396-8	Q09352-02	80	10	5	5	20	10	5	35	Lung Adenocarcinoma. Non-Small Cell Lung Carcinoma (NSCLC).	-
3	**Lung SCC**	QMTB398-1	Q06961-01	50	5	15	30	50	45	30	125	Lung Squamous Cell Carcinoma (SCC) with extensive necrosis. NSCLC.	-
3	**Lung SCC**	QMTB398-2	Q06971-01	5	10	50	35	95	85	35	215	Lung Squamous Cell Carcinoma (SCC) nonkeratinizing with localized clear cell. NSCLC.	-
3	**Lung SCC**	QMTB398-3	Q04038-01	95	5	0	0	5	0	0	5	Lung Squamous Cell Carcinoma (SCC). NSCLC.	-
3	**Lung SCC**	QMTB398-4	Q06962-06	0	20	30	50	100	80	50	230	Lung Squamous Cell Carcinoma (SCC) nonkeratinizing. NSCLC.	-
3	**Lung SCC**	QMTB398-5	Q06964-02	0	0	30	70	100	100	70	270	Lung Squamous Cell Carcinoma (SCC) focally keratinizing. NSCLC.	-
3	**Lung SCC**	QMTB398-6	Q06963-03	100	0	0	0	0	0	0	0	Lung SCC with extensive necrosis, nonkeratinizing, focal clear cell. NSCLC.	-
3	**Lung SCC**	QMTB398-7	Q06967-03	0	70	0	30	100	30	30	160	Lung Squamous Cell Carcinoma (SCC). NSCLC.	Paucity of tumor cells.
3	**Lung SCC**	QMTB398-8	Q06966-02	0	20	30	50	100	80	50	230	Lung Squamous Cell Carcinoma (SCC) focally keratinizing. NSCLC.	Maturational dependent signal in tumor. Fixation artifact.
3	**Lung SCC**	QMTB398-9	Q06965-01	5	20	15	60	95	75	60	230	Lung Squamous Cell Carcinoma (SCC) focally keratinizing. NSCLC.	-
4	**TNBC**	QMTB232-3	Q03954	10	10	30	50	90	80	50	220	Triple Negative Breast Cancer (TNBC). Ductal Cell Carcinoma. Her2-, ER-, and PR-.	Finely granular cytoplasmic reactivity, predominately at nuclear membrane.
4	**TNBC**	QMTB232-5	Q03957	100	0	0	0	0	0	0	0	Triple Negative Breast Cancer (TNBC). Ductal Cell Carcinoma, Tubular. Her2-, ER-, and PR-.	-
4	**TNBC**	QMTB232-6	Q03966	100	0	0	0	0	0	0	0	Triple Negative Breast Cancer (TNBC). Ductal Cell Carcinoma. Her2-, ER-, and PR-.	-
5	**TNBC**	Q09250	Q09250	100	0	0	0	0	0	0	0	Triple Negative Breast Cancer (TNBC). Infiltrating Ductal Cell Carcinoma. Her2-, ER-, and PR-.	-
6	**TNBC**	Q09256	Q09256	100	0	0	0	0	0	0	0	Triple Negative Breast Cancer (TNBC). Infiltrating Ductal Cell Carcinoma. Her2-, ER-, and PR-.	-
1	**Breast Cancer**	QMTB425-03-1	Q01420-01	100	0	0	0	0	0	0	0	Infiltrating Ductal Breast Cancer (non-TNBC).	-
1	**Breast Cancer**	QMTB425-03-2	Q05065-03	0	20	50	30	100	80	30	210	Infiltrating Ductal Breast Cancer (non-TNBC).	Distinct submembrane and nuclear membrane-associated staining pattern.
1	**Breast Cancer**	QMTB425-03-3	Q01425-02	0	35	40	25	100	65	25	190	Infiltrating Ductal Breast Cancer (non-TNBC).	Submembrane and nuclear membrane-associated staining pattern.
1	***Breast Cancer***	*QMTB425-03-4*	*Q01422-01*	*0*	*0*	*20*	*80*	*100*	*100*	*80*	*280*	*Ductal Papillary Breast Cancer. Not confirmed as non-TNBC (or hormone receptor, HR, +).*	*Submembrane and nuclear membrane-associated staining pattern. Not confirmed HR+.*
1	**Breast Cancer**	QMTB425-03-5	Q01421-01	0	10	40	50	100	90	50	240	Infiltrating Ductal Breast Cancer (non-TNBC).	Pattern predominantly nuclear membrane-associated.
1	***Breast Cancer***	*QMTB425-03-6*	*Q01412-03*	*0*	*0*	*0*	*100*	*100*	*100*	*100*	*300*	*Lobular Breast Cancer. Not confirmed as non-TNBC (or hormone receptor, HR, +).*	*Lobular invasive breast cancer. Not confirmed HR+.*
1	**Breast Cancer**	QMTB425-03-7	Q01424-01	0	40	40	20	100	60	20	180	Infiltrating Ductal Breast Cancer (non-TNBC).	Pattern predominantly submembrane.
50	**Pancreatic Cancer**	QMTB295-1	Q07471	40	10	30	20	60	50	20	130	Pancreatic Adenocarcinoma.	Poorly differentiated. Geographic necrosis. Polar perinuclear adluminal pattern.
50	**Pancreatic Cancer**	QMTB295-2	Q07472	100	0	0	0	0	0	0	0	Pancreatic Adenocarcinoma.	Poorly differentiated.
50	**Pancreatic Cancer**	QMTB295-3	Q07473	100	0	0	0	0	0	0	0	Pancreatic Carcinoma.	Paucity of tumor cells. Extensive necrosis.
50	**Pancreatic Cancer**	QMTB295-4	Q07475	5	15	15	65	95	80	65	240	Pancreatic Adenocarcinoma with intestine.	Well differentiated. Predominately adluminal staining pattern.
50	**Pancreatic Cancer**	QMTB295-5	Q07476	0	0	10	90	100	100	90	290	Mucinous Pancreatic Adenocarcinoma.	Mucinous tumor.
50	**Pancreatic Cancer**	QMTB295-6	Q07477	100	0	0	0	0	0	0	0	Pancreatic Adenocarcinoma. Signet ring features; possible neuroendocrine.	Acinar cell tumor.
50	**Pancreatic Cancer**	QMTB295-7	Q07478	0	70	30	0	100	30	0	130	Pancreatic Adenocarcinoma.	Moderately differentiated.

Table 1. (Continued)

Sensitivity Screen for CCR5 Human IgG4a in Various Tumors														
Sample Information				CCR5 Cytoplasmic Tumor Staining								Additional Sample Information	Comments
				% at Differential Intensities				% Staining ≥1+	% Staining ≥2+	% Staining ≥3+	H-Score		
Slide #	Tissue Type	Q/MTB#	Sample ID	0	1+	2+	3+					Pathology	
8	**Urothelial Cancer**	QMTB360-1	Q03633	75	10	5	10	25	15	10	50	Bladder Cancer. Transitional Cell.	Poorly differentiated. Lymphocyte population diffusely/weakly stains for CCR5.
8	***Urothelial Cancer***	*QMTB360-2*	*Q03635-13*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*Bladder Cancer. Papillary Transitional Cell (bladder & ulceration).*	*No evaluable tumor.*
8	**Urothelial Cancer**	QMTB360-3	Q03634	80	0	10	10	20	20	10	50	Bladder Cancer. Transitional Cell (in situ in mucosa).	Poorly differentiated. Subset of lymphocytes are CCR5 reactive.
8	***Urothelial Cancer***	*QMTB360-4*	*Q06083*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*Bladder Cancer. Transitional Cell.*	*No evaluable tumor.*
8	**Urothelial Cancer**	QMTB360-5	Q08952	0	0	0	100	100	100	100	300	Bladder Cancer. Papillary Transitional Cell.	-
8	**Urothelial Cancer**	QMTB360-6	Q08955	0	10	70	20	100	90	20	210	Bladder Cancer. Papillary, Urinary.	Apical submembranous staining pattern.
8	***Urothelial Cancer***	*QMTB360-7*	*Q08956*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*NET*	*Bladder Cancer. Transitional Cell.*	*No evaluable tumor.*
8	**Urothelial Cancer**	QMTB360-8	Q08958	0	50	10	40	100	50	40	190	Bladder Cancer. Papillary Transitional Cell.	Well differentiated.
8	**Urothelial Cancer**	QMTB360-9	Q08959	0	0	0	100	100	100	100	300	Bladder Cancer. Papillary Transitional Cell.	-
9	**Colorectal Cancer**	QMTB426-1	Q1192-01	0	5	30	65	100	95	65	260	Adenocarcinoma w/mucinous components.	Plasma membrane staining pattern present.
9	***Colorectal Cancer***	*QMTB426-2*	*Q1194-05*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*Mucinous tumor.*	*Mucinous, clear cell tumor type makes staining interpretation difficult. Not scored.*
9	**Colorectal Cancer**	QMTB426-3	Q5123-02	0	0	0	100	100	100	100	300	Mucinous tumor.	Predominately cytoplasmic staining pattern.
9	**Colorectal Cancer**	QMTB426-4	Q2874-01	100	0	0	0	0	0	0	0	Adenocarcinoma of cecum.	-
9	**Colorectal Cancer**	QMTB426-5	Q3116-02	50	15	30	5	50	35	5	90	Adenocarcinoma.	Uneven fixation.
9	**Colorectal Cancer**	QMTB426-6	Q2872-02	30	60	10	0	70	10	0	80	Adenocarcinoma.	Well differentiated.
9	**Colorectal Cancer**	QMTB426-7	Q2863-01	15	60	10	15	85	25	15	125	Adenocarcinoma.	Moderately to well differentiated.
9	**Colorectal Cancer**	QMTB426-8	Q2875-02	75	8	15	2	25	17	2	44	Adenocarcinoma.	Moderately differentiated.
9	**Colorectal Cancer**	QMTB426-9	Q5123-03	15	80	5	0	85	5	0	90	Same patient as QMTB426-3 but different sample block.	Metastatic to LN.
9	**Colorectal Cancer**	QMTB426-10	Q2138-01	5	15	20	60	95	80	60	235	Adenocarcinoma of rectum.	Plasma membrane staining pattern present.
10	**H&N Cancer**	QMTB419-01-1	Q03655-01	90	7	2	1	10	3	1	14	Head and Neck (H&N) Parotid Cancer.	Cytoplasmic/perinuclear staining pattern.
10	**H&N Cancer**	QMTB419-01-2	Q03805-01	100	0	0	0	0	0	0	0	Head and Neck (H&N) Laryngeal Cancer.	Non-specific labeling in keratinous material observed.
10	**H&N Cancer**	QMTB419-01-3	Q03806-01	70	20	10	0	30	10	0	40	Head and Neck (H&N) Laryngeal Cancer.	-
10	**H&N Cancer**	QMTB419-01-4	Q03807	5	10	30	55	95	85	55	235	Head and Neck (H&N) Laryngeal Cancer.	Maturational dependent signal in tumor. Fixation artifact.
10	**H&N Cancer**	QMTB419-01-5	Q03678-02	100	0	0	0	0	0	0	0	Head and Neck (H&N) Parotid Cancer.	-
10	**H&N Cancer**	QMTB419-01-6	Q03810	10	20	40	30	90	70	30	190	Head and Neck (H&N) Laryngeal Cancer.	Predominately nuclear membrane-associated pattern.
10	**H&N Cancer**	QMTB419-01-7	Q03812-04	100	0	0	0	0	0	0	0	Head and Neck (H&N) Laryngeal Cancer.	-
10	***H&N Cancer***	*QMTB419-01-8*	*Q06780*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*Head and Neck (H&N) Laryngeal Cancer.*	*Fixation artifact. Not scored.*
11	***Sarcoma***	*QMTB312-1*	*Q06920*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*Desmoplastic Small Round Cell Tumor (DSRCT).*	*Fixation artifact and paucity of tumor cells. Not scored.*
11	**Sarcoma**	QMTB312-2	Q06923	0	90	5	5	100	10	5	115	Pleomorphic Rhabdomyosarcoma.	-
11	**Sarcoma**	QMTB312-3	Q06924	52	40	5	3	48	8	3	59	Biphasic Synovial Sarcoma.	-
11	**Sarcoma**	QMTB312-4	Q06925	100	0	0	0	0	0	0	0	Ewing's Sarcoma.	Decalcified.
11	**Sarcoma**	QMTB312-5	Q06927	100	0	0	0	0	0	0	0	Biphasic Synovial Sarcoma.	-
11	**Sarcoma**	QMTB312-6	Q06929	100	0	0	0	0	0	0	0	Alveolar Rhabdomyosarcoma.	Non-specific reactivity observed in artifact.
11	**Sarcoma**	QMTB312-7	Q06930	95	5	0	0	5	0	0	5	Biphasic Synovial Sarcoma.	-
11	**Sarcoma**	QMTB312-8	Q06931	90	10	0	0	10	0	0	10	Desmoplastic Small Round Cell Tumor (DSRCT).	Significant artifact present.
11	**Sarcoma**	QMTB312-9	Q06934	100	0	0	0	0	0	0	0	Rhabdomyosarcoma.	-

Figure 1. CCR5 Staining in Lung Adeno, Lung SCC, & TNBC

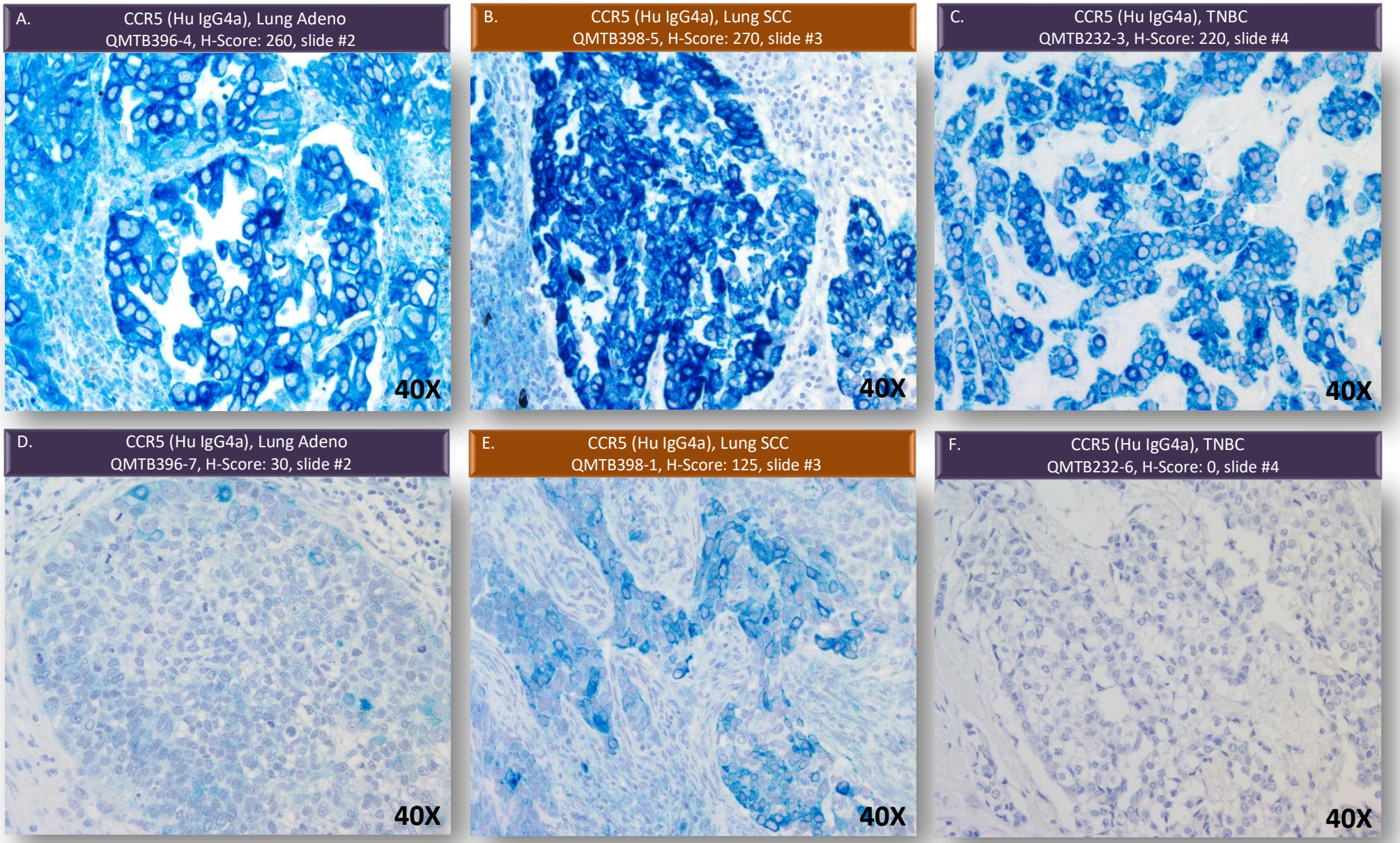


Figure 2. CCR5 Staining in Breast, Pancreatic, & Urothelial Cancer

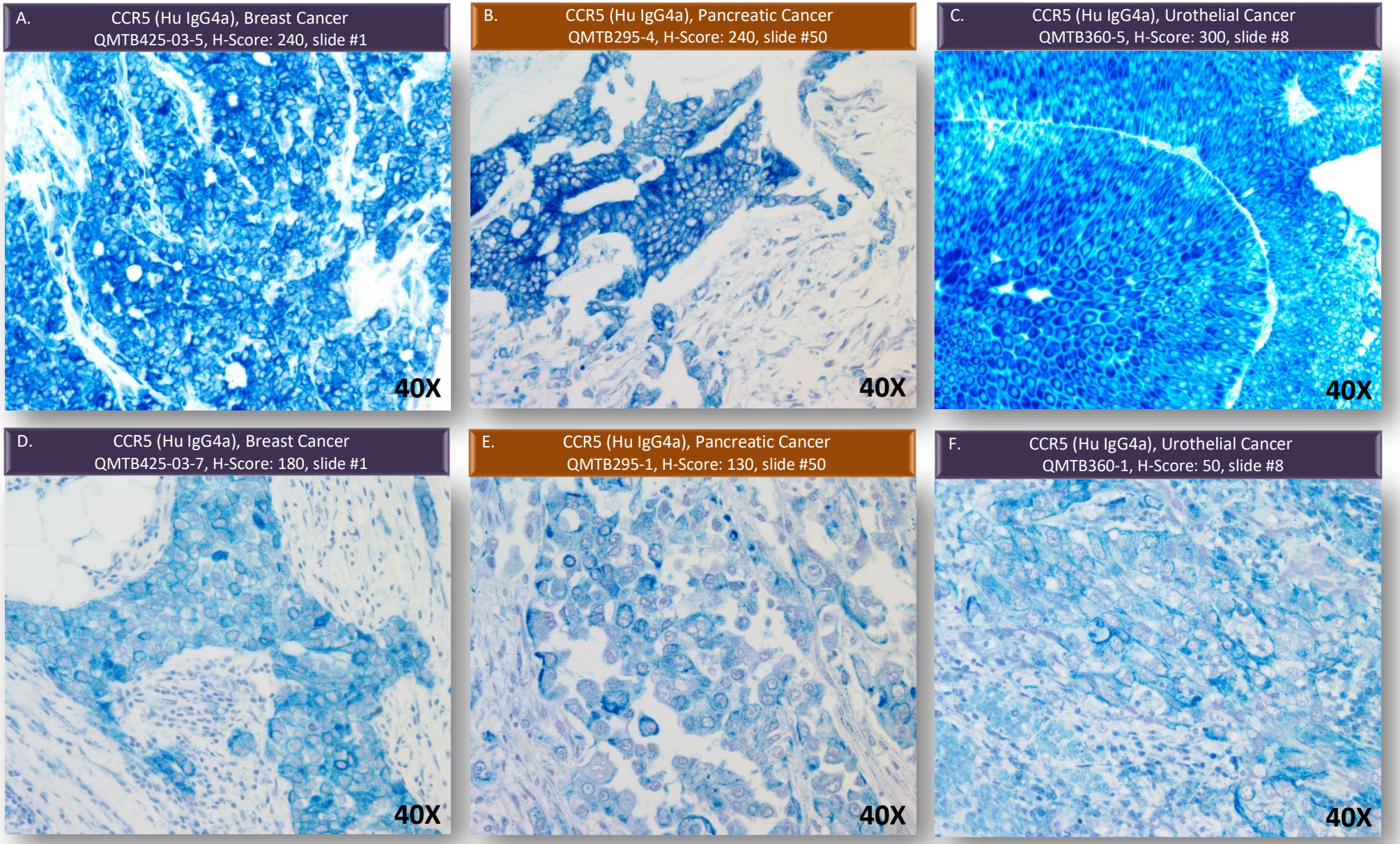


Figure 3. CCR5 Staining in Colorectal Cancer, H&N Cancer, & Sarcoma

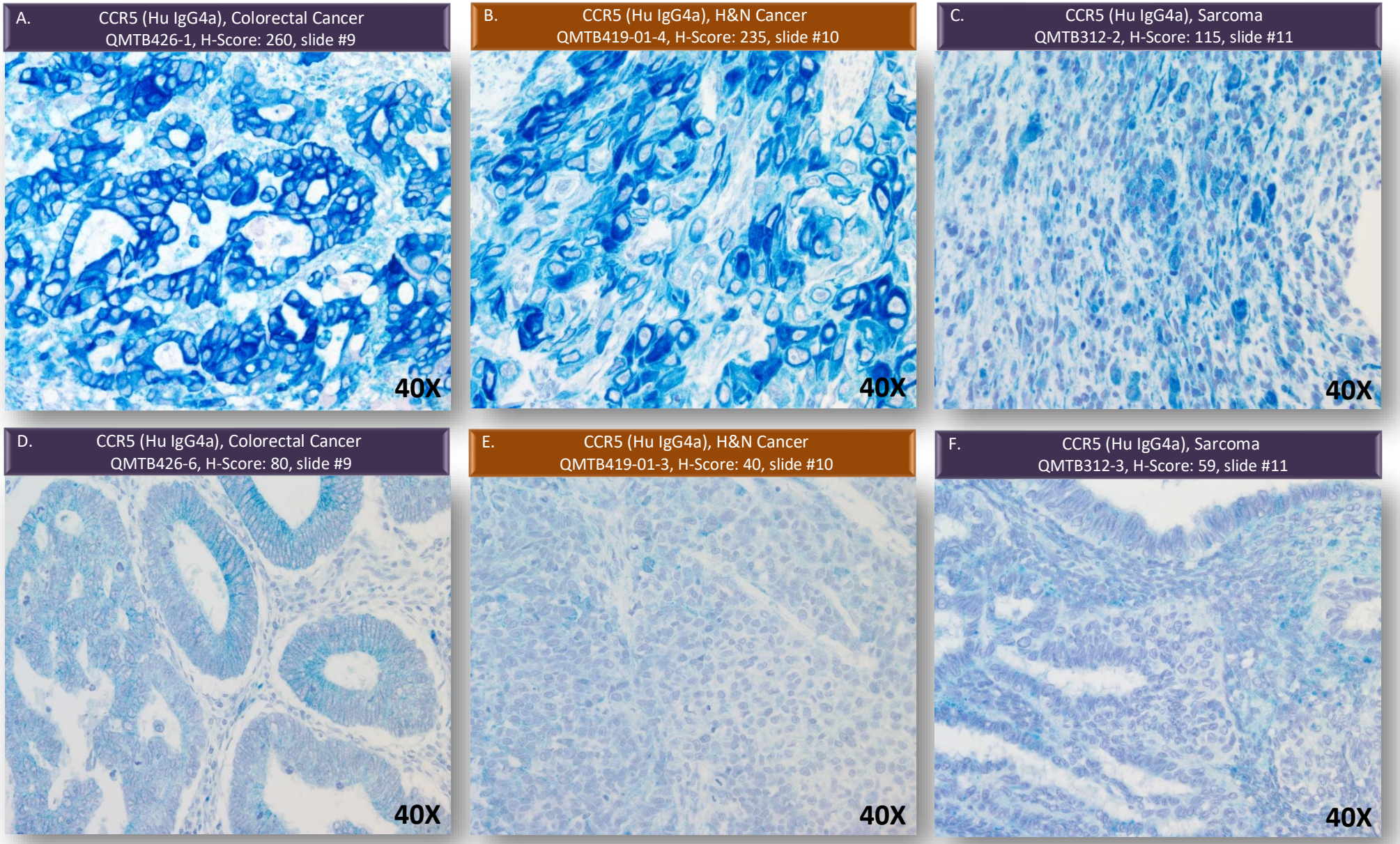
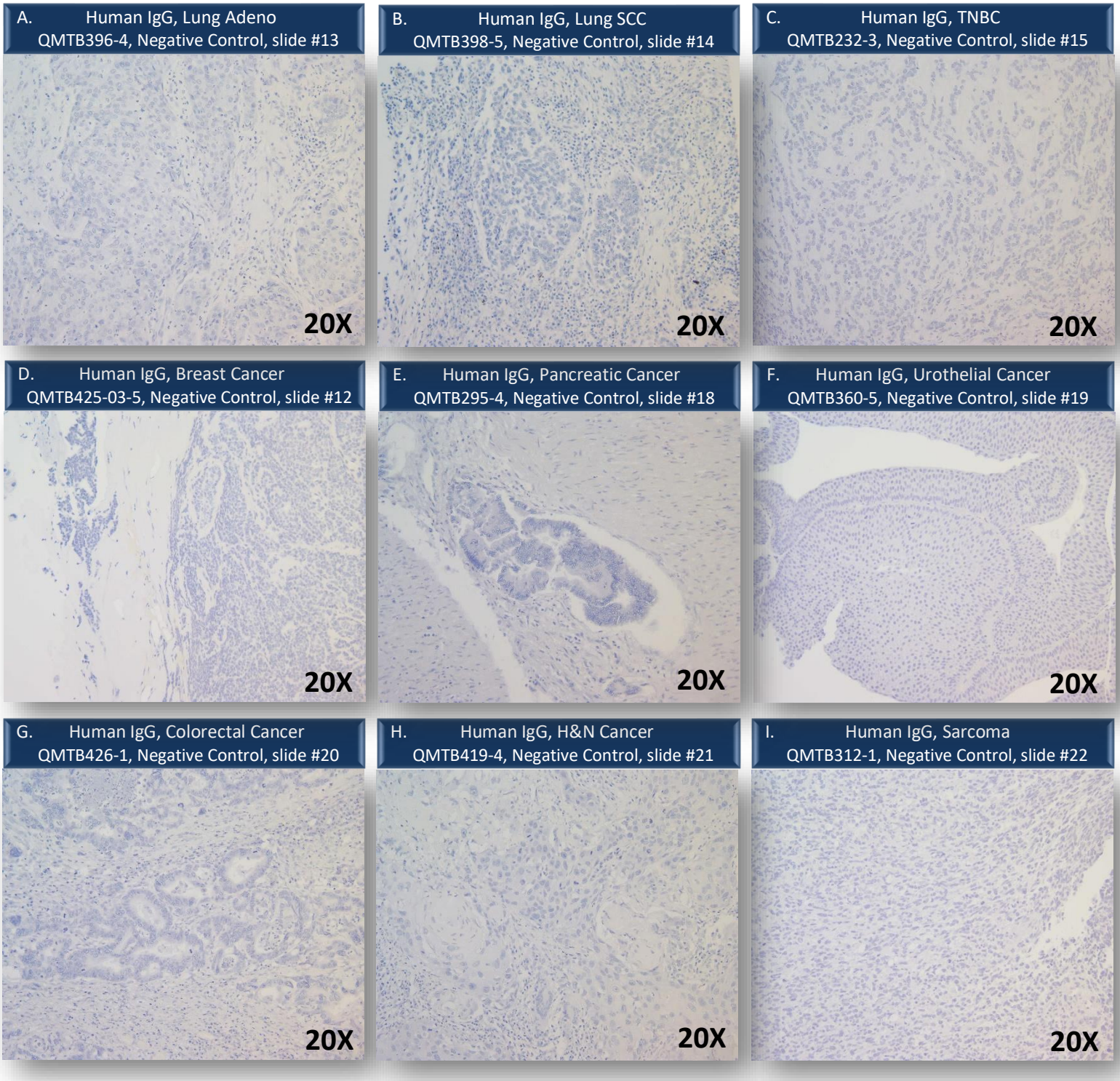
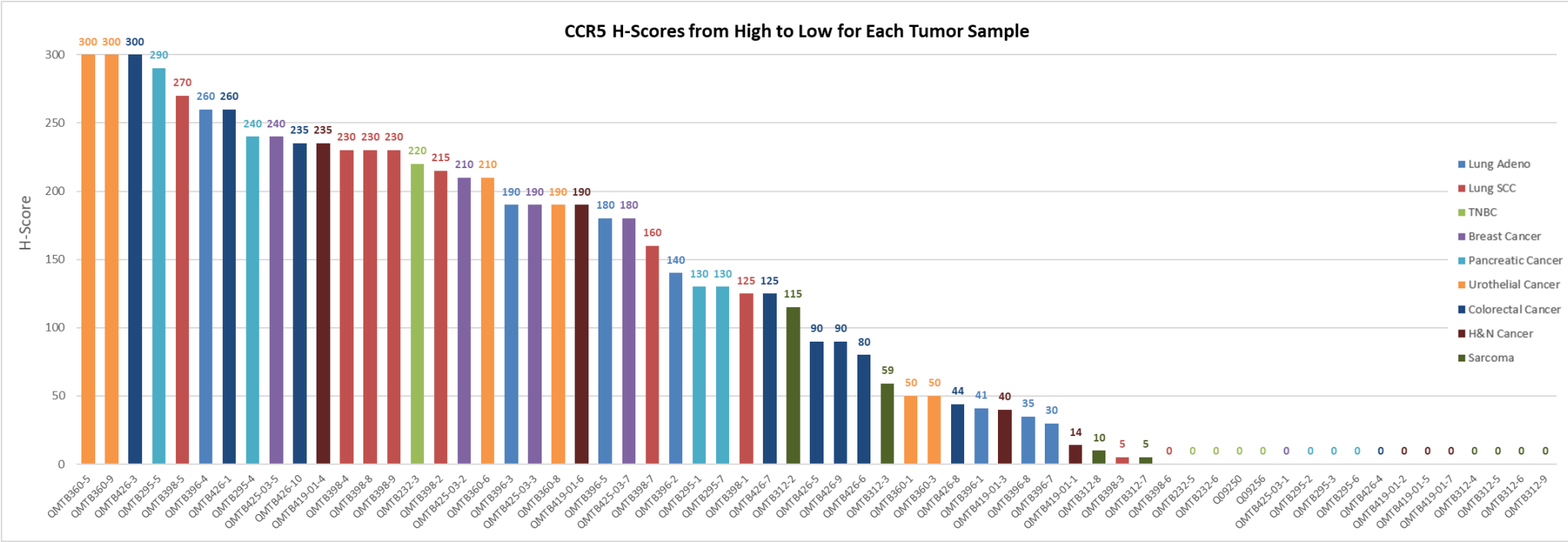


Figure 4. Representative Human IgG Isotype-Match Controls



Graph 1. Distribution of CCR5 H-Scores by Tumor Sample



Graph 2. Average H-Scores for CCR5 by Cancer Indication

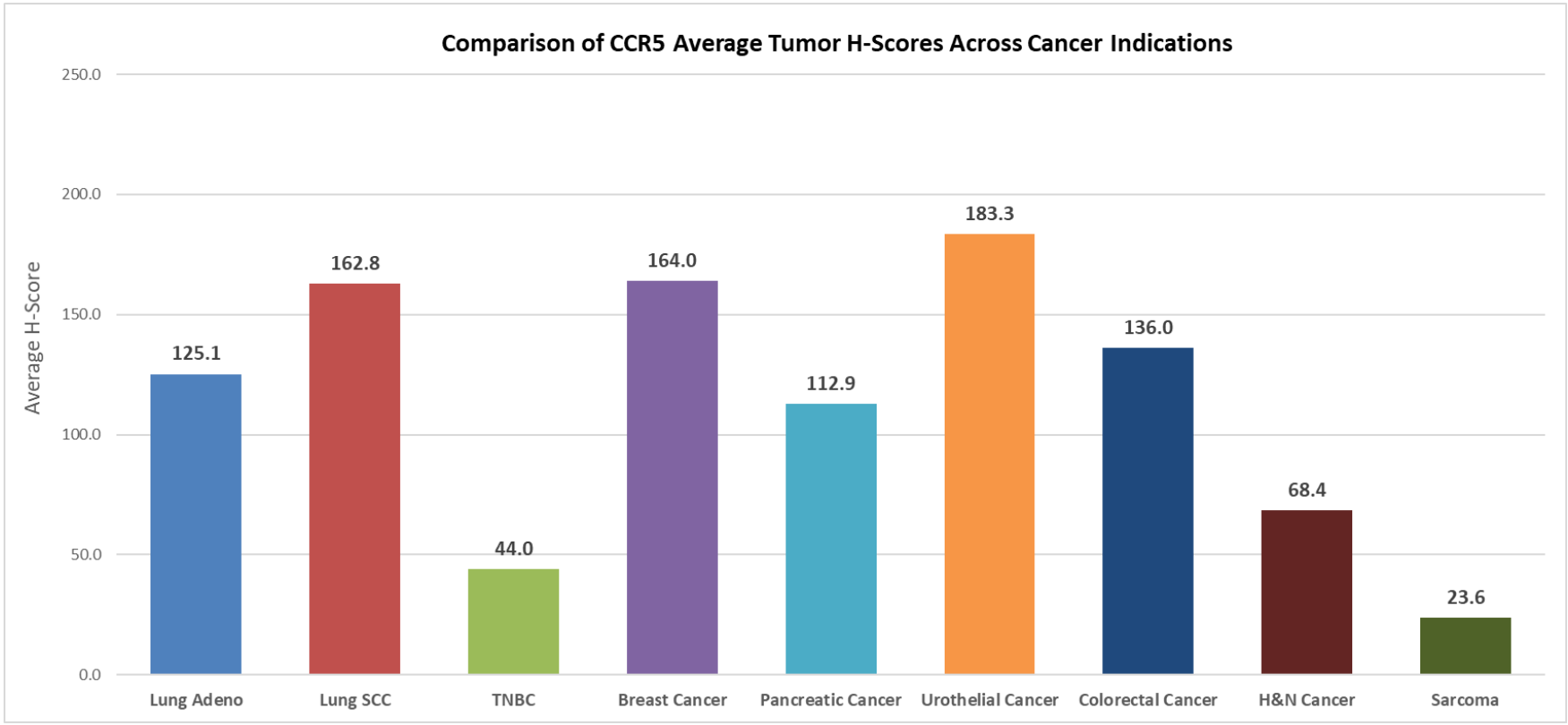


Table 2. CCR5 Reactivity in Tumor Types by Thresholds of ≥1+ Staining

Breakdown of CCR5 Tumor Reactivity Data at Intensities ≥1+													
Tumor Type	Evaluable Cases	≥1+ Staining in ≥1% Tumor Cells		≥1+ Staining in ≥10% Tumor Cells		≥1+ Staining in ≥25% Tumor Cells		≥1+ Staining in ≥50% Tumor Cells		≥1+ Staining in ≥75% Tumor Cells		≥1+ Staining in ≥90% Tumor Cells	
		No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication
Lung Adeno	7	7	100%	7	100%	5	71%	4	57%	4	57%	4	57%
Lung SCC	9	8	89%	7	78%	7	78%	7	78%	6	67%	6	67%
TNBC	5	1	20%	1	20%	1	20%	1	20%	1	20%	1	20%
Breast Cancer	5	4	80%	4	80%	4	80%	4	80%	4	80%	4	80%
Pancreatic Cancer	7	4	57%	4	57%	4	57%	4	57%	3	43%	3	43%
Urothelial Cancer	6	6	100%	6	100%	5	83%	4	67%	4	67%	4	67%
Colorectal Cancer	9	8	89%	8	89%	8	89%	7	78%	5	56%	3	33%
H&N Cancer	7	4	57%	4	57%	3	43%	2	29%	2	29%	2	29%
Sarcoma	8	4	50%	3	38%	2	25%	1	13%	1	13%	1	13%

Table 3. CCR5 Reactivity in Tumor Types by Thresholds of ≥2+ Staining

Breakdown of CCR5 Tumor Reactivity Data at Intensities ≥2+													
Tumor Type	Evaluable Cases	≥2+ Staining in ≥1% Tumor Cells		≥2+ Staining in ≥10% Tumor Cells		≥2+ Staining in ≥25% Tumor Cells		≥2+ Staining in ≥50% Tumor Cells		≥2+ Staining in ≥75% Tumor Cells		≥2+ Staining in ≥90% Tumor Cells	
		No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication
Lung Adeno	7	7	100%	6	86%	4	57%	2	29%	1	14%	1	14%
Lung SCC	9	7	78%	7	78%	7	78%	5	56%	5	56%	1	11%
TNBC	5	1	20%	1	20%	1	20%	1	20%	1	20%	0	0%
Breast Cancer	5	4	80%	4	80%	4	80%	4	80%	2	40%	1	20%
Pancreatic Cancer	7	4	57%	4	57%	4	57%	3	43%	2	29%	1	14%
Urothelial Cancer	6	6	100%	6	100%	4	67%	4	67%	3	50%	3	50%
Colorectal Cancer	9	8	89%	7	78%	5	56%	3	33%	3	33%	2	22%
H&N Cancer	7	4	57%	3	43%	2	29%	2	29%	1	14%	0	0%
Sarcoma	8	2	25%	1	13%	0	0%	0	0%	0	0%	0	0%

Table 4. CCR5 Reactivity in Tumor Types by Thresholds of ≥3+ Staining

Breakdown of CCR5 Tumor Reactivity Data at Intensities ≥3+													
Tumor Type	Evaluable Cases	≥3+ Staining in ≥1% Tumor Cells		≥3+ Staining in ≥10% Tumor Cells		≥3+ Staining in ≥25% Tumor Cells		≥3+ Staining in ≥50% Tumor Cells		≥3+ Staining in ≥75% Tumor Cells		≥3+ Staining in ≥90% Tumor Cells	
		No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication
Lung Adeno	7	7	100%	4	57%	3	43%	1	14%	0	0%	0	0%
Lung SCC	9	7	78%	7	78%	7	78%	4	44%	0	0%	0	0%
TNBC	5	1	20%	1	20%	1	20%	1	20%	0	0%	0	0%
Breast Cancer	5	4	80%	4	80%	3	60%	1	20%	0	0%	0	0%
Pancreatic Cancer	7	3	43%	3	43%	2	29%	2	29%	1	14%	1	14%
Urothelial Cancer	6	6	100%	6	100%	3	50%	2	33%	2	33%	2	33%
Colorectal Cancer	9	6	67%	4	44%	3	33%	3	33%	1	11%	1	11%
H&N Cancer	7	3	43%	2	29%	2	29%	1	14%	0	0%	0	0%
Sarcoma	8	2	25%	0	0%	0	0%	0	0%	0	0%	0	0%

Table 5. CCR5 Reactivity in Tumor Types by H-Score Breakdowns

Breakdown of CCR5 Tumor Reactivity Data by H-Scores														
Tumor Type	Evaluable Cases	H-Score ≥1		H-Score ≥50		H-Score ≥100		H-Score ≥150		H-Score ≥200		H-Score ≥250		Average H-Score
		No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	No. of Cases	% of Indication	
Lung Adeno	7	7	100%	4	57%	4	57%	3	43%	1	14%	1	14%	125.1
Lung SCC	9	8	89%	7	78%	7	78%	6	67%	5	56%	1	11%	162.8
TNBC	5	1	20%	1	20%	1	20%	1	20%	1	20%	0	0%	44.0
Breast Cancer	5	4	80%	4	80%	4	80%	4	80%	2	40%	0	0%	164.0
Pancreatic Cancer	7	4	57%	4	57%	4	57%	2	29%	2	29%	1	14%	112.9
Urothelial Cancer	6	6	100%	6	100%	4	67%	4	67%	3	50%	2	33%	183.3
Colorectal Cancer	9	8	89%	7	78%	4	44%	3	33%	3	33%	2	22%	136.0
H&N Cancer	7	4	57%	2	29%	2	29%	2	29%	1	14%	0	0%	68.4
Sarcoma	8	4	50%	2	25%	1	13%	0	0%	0	0%	0	0%	23.6

3b. CCR5 Precision & Reproducibility

Intra- & Inter-Assay Staining Results

The results from the sensitivity screen helped identify appropriate tissues to use in determining the precision and reproducibility of the IHC assay for CCR5. For this validation testing, 4 samples were selected that included a range of high, moderate, low, and negative expression across the Colorectal Carcinoma (CRC) and Lung Squamous Cell Carcinoma (SCC) indications.

The following samples with their relative levels of CCR5 expression (as determined during sensitivity testing) were selected for validation testing: Colorectal Cancer- QMTB426-3 (high), Lung SCC- QMTB398-2 (moderate), Colorectal Cancer- QMTB426-6 (low), and Lung SCC- QMTB398-6 (negative).

Using the IHC assay, these samples were run in triplicate within one run for precision testing. In a separate run, the same samples were run in triplicate by a second operator for reproducibility testing. Replicate slides were prepared as serial sections (one section of tissue per slide with minimal loss of material between preparations).

In other words, intra-assay (precision) and inter-assay (reproducibility) were determined using a 2-run series with 3 replicate sections (per run) of each of the 4 selected samples for the CCR5 biomarker. Two trained operators ran the CCR5 assay using different TechMate instruments (Operator 1, Run 1; Operator 2, Run 2). Appropriate positive and negative controls were included in each staining run.

All CCR5 replicates stained during validation were reviewed and scored by Stanford's Board-certified pathologist using the H-Score approach (**Section 2**) for cytoplasmic staining in tumor cells to determine precision and reproducibility. The replicates were deemed acceptable by the pathologist as compared to the section stained with CCR5 during sensitivity for each sample. Full validation scoring, including statistical analysis for H-Score replicate sets, are shown in **Table 6**.

Similar cellular patterns of CCR5 reactivity were observed in all replicates in the pattern, percent, and intensity of cytoplasmic staining. This consistency is represented in **Figures 5** & **6**. Any minor or graded changes observed in staining between replicates were attributable to slight changes in the amount of tumor in each serial section. Images of corresponding Rabbit IgG negative controls are included in the bottom row of these figures.

Statistical Analysis of H-Scores

Acceptance of validation is determined through evaluation of consistency in staining patterns and semi-quantitative scores with the general criteria that the standard error of the mean (SEM) among replicates does not exceed 5, and the coefficient of variation (CV) does not exceed 20% (for samples with H-Score values ≥50).

Summarized validation scoring results for CCR5 H-Score analysis including the mean, standard deviation (Std Dev), standard error of the mean (SEM), and coefficient of variation (CV) for each set of 6 replicates are presented in **Table 7.**

Acceptance criteria were met for the CCR5 (Hu IgG4a) IHC assay with a standard deviation (Std Dev) that did not exceed 6.8, a standard error of the mean (SEM) that did not exceed 2.8, and a coefficient of variation (CV) did not exceed 8% for all sample sets. As such, the CCR5 IHC assay for detection in human cancers can be considered successfully validated, as determined by Stanford's board-certified pathologist.

Table 6. Precision & Reproducibility of IHC Assay for CCR5

CCR5 Precision & Reproducibility Testing with H-Score Analysis														
Run/ Operator	Sample Information				Tumor Cell Staining Differential Intensities and H-Score					Statistical Analysis of H-Scores				Comments
	Tissue Type	Q/MTB#	Replicate	Slide#	0	1+	2+	3+	H-Score	H-Score Mean	H-Score Std Dev	H-Score SEM	H-Score CV (%)	
1	Colorectal Cancer	QMTB426-3	1	27	0	0	0	100	**300**	**300.0**	**0.0**	**0.0**	**0%**	-
			2	28	0	0	0	100	**300**					
			3	29	0	0	0	100	**300**					
2	Colorectal Cancer	QMTB426-3	1	42	0	0	0	100	**300**					
			2	43	0	0	0	100	**300**					
			3	44	0	0	0	100	**300**					
1	Lung SCC	QMTB398-2	1	31	5	10	50	35	**215**	**209.2**	**4.9**	**2.0**	**2%**	For slide #47, edge of tissue nearest label not scored due to fixation artifact.
			2	32	10	5	50	35	**210**					
			3	33	10	5	55	30	**205**					
2	Lung SCC	QMTB398-2	1	46	0	15	55	30	**215**					
			2	47	5	15	50	30	**205**					
			3	48	5	15	50	30	**205**					
1	Colorectal Cancer	QMTB426-6	1	27	30	60	10	0	**80**	**81.7**	**6.8**	**2.8**	**8%**	-
			2	28	25	60	15	0	**90**					
			3	29	25	60	15	0	**90**					
2	Colorectal Cancer	QMTB426-6	1	42	30	60	10	0	**80**					
			2	43	30	65	5	0	**75**					
			3	44	30	65	5	0	**75**					
1	Lung SCC	QMTB398-6	1	31	100	0	0	0	**0**	**0.0**	**0.0**	**0.0**	**0%**	-
			2	32	100	0	0	0	**0**					
			3	33	100	0	0	0	**0**					
2	Lung SCC	QMTB398-6	1	46	100	0	0	0	**0**					
			2	47	100	0	0	0	**0**					
			3	48	100	0	0	0	**0**					

Table 7. Summary of CCR5 Precision & Reproducibility

Precision & Reproducibility Summary for CCR5 H-Scores												
Biomarker	Tissue Type	Q/MTB#	Operator 1			Operator 2			H-Score Mean	H-Score Std Dev	H-Score SEM	H-Score CV
			Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3				
CCR5 Tumor Staining	Colorectal Cancer	QMTB426-3	300	300	300	300	300	300	**300.0**	**0.0**	**0.0**	**0%**
	Lung SCC	QMTB398-2	215	210	205	215	205	205	**209.2**	**4.9**	**2.0**	**2%**
	Colorectal Cancer	QMTB426-6	80	90	90	80	75	75	**81.7**	**6.8**	**2.8**	**8%**
	Lung SCC	QMTB398-6	0	0	0	0	0	0	**0.0**	**0.0**	**0.0**	**0%**

Figure 5. Precision & Reproducibility of CCR5 in Lung SCC QMTB398-2

Lung SCC, QMTB398-2, Top Three Rows: CCR5, Bottom Row: Rabbit IgG Negative Control, 40X

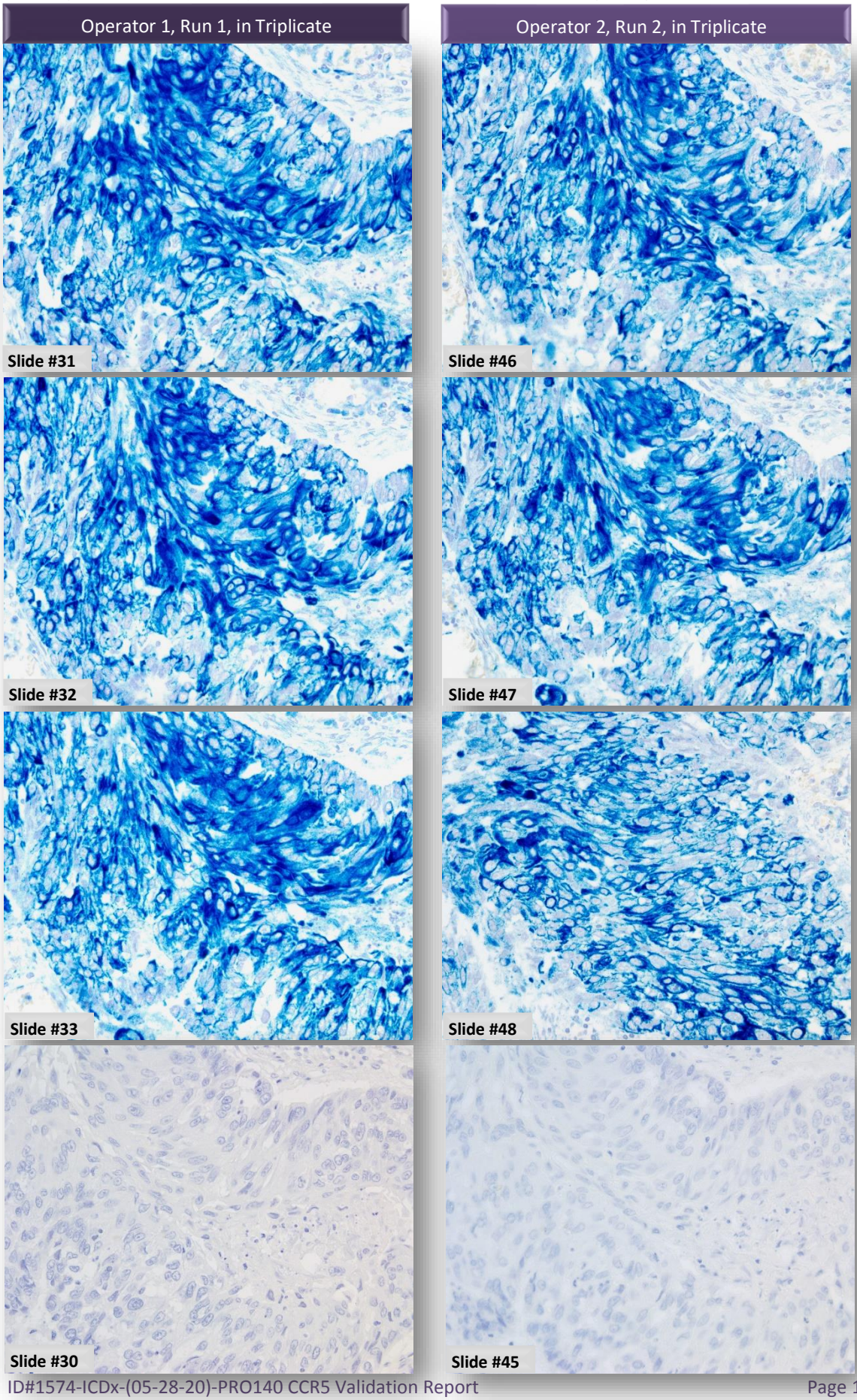
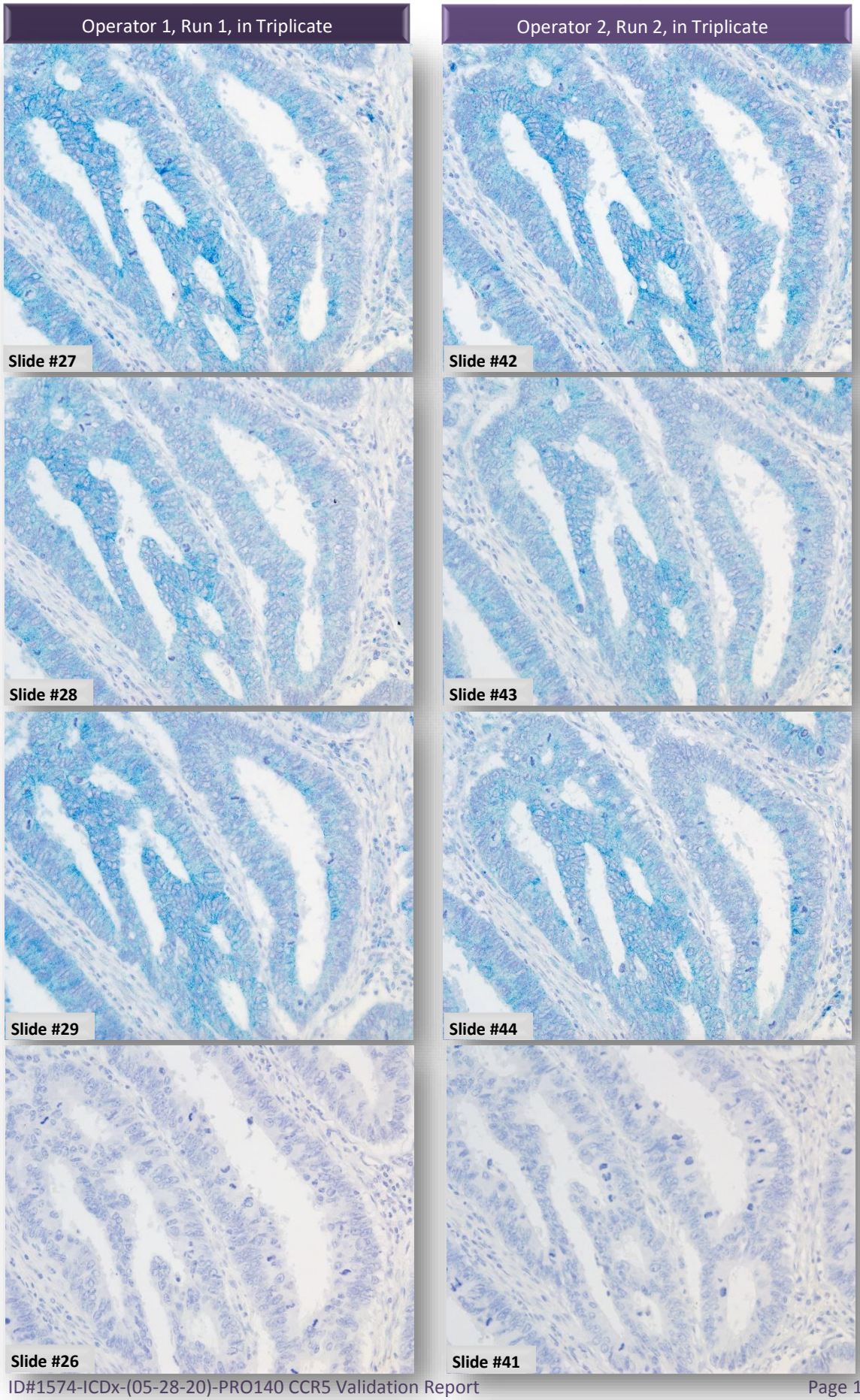


Figure 6. Precision & Reproducibility of CCR5 in Colorectal Cancer QMTB426-6

Colorectal Cancer, QMTB426-6, Top Three Rows: CCR5, Bottom Row: Rabbit IgG Negative Control, 40X



8. Study Personnel

	Name	Hardcopy Signature and Date
Study Director	Bruce Patterson	
VP of Development	Amruta Pise	
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