OmniSeq®
700 Ellicott Street | Buffalo NY, 14203

Identifying major immune subsets of GI tumors for clinical purposes

The right drug or right trial...
For Every Patient

Early A[1,#], Pabla S[1], Conroy JM[1,2], Nesline MK[1], Glenn S[1,2], Papanicolau-Sengos A[1], Burgher B[1], Giamo V[1], Andreas J[1], DePietro P[1], Lenzo F[1], Wang Y[1], Morrison CD[1,2*]
OmniSeq, Inc[1], Buffalo, NY; Roswell Park Comprehensive Cancer Center[2], Buffalo, NY # Amy.Early@RoswellPark.org; *Carl.Morrison@OmniSeq.com

Introduction

Microsatellite stable (MSS) gastrointestinal (GI) tumors, including colorectal and non-colorectal, have demonstrated low response rates to immune checkpoint inhibitors (ICIs).

Currently, there is uncertainty as to how to evaluate MSS GI tumors for evidence of checkpoint blockade, as PD-L1 immunohistochemistry (IHC) has demonstrated limited utility for these tumors.

Understanding other mechanisms of immunosuppression in GI tumors, such as myeloid or metabolic suppression and the degree of CD8+ T-cell infiltration, may illuminate immunotherapy selection in this patient population.

Methods

Formalin fixed paraffin embedded (FFPE) tissue from 127 MSI stable (MSS) GI tumors, including colorectal (n=80), pancreatic (n=40) and small bowel (n=7) were evaluated for PD-L1 expression by immunohistochemistry (IHC) using the 22c3 pharmDx antibody on Autostainer Link 48 (Agilent, Santa Clara, CA). Tumor proportion score (TPS) was interpreted per published guidelines.

RNA was extracted to perform gene expression profiling (GEX) by RNA-Sequencing of 54 immune-related genes, including targets of immunomodulatory immunotherapeutics that are currently FDA approved (i.e., PD-L1, PD-1) or in clinical development (i.e., CSF1R, VISTA), as well as subsets of tumor infiltrating lymphocytes (TILs), including CD8.

Normalized GEX values (nRPM) were ranked from 0 to 100 and compared to a reference population of 167 cases that had a broad range of immune gene expression in multiple tumor types.

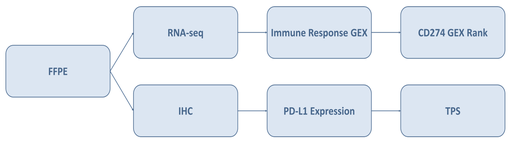
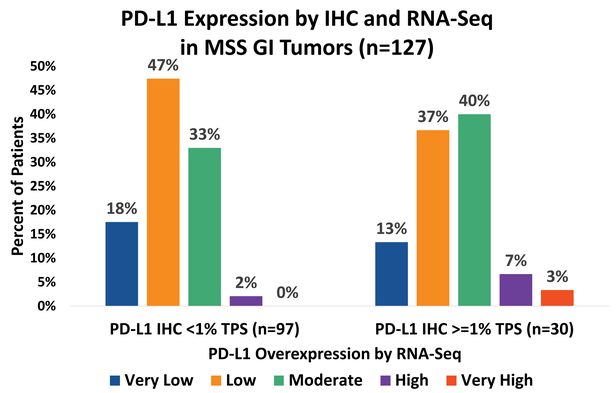


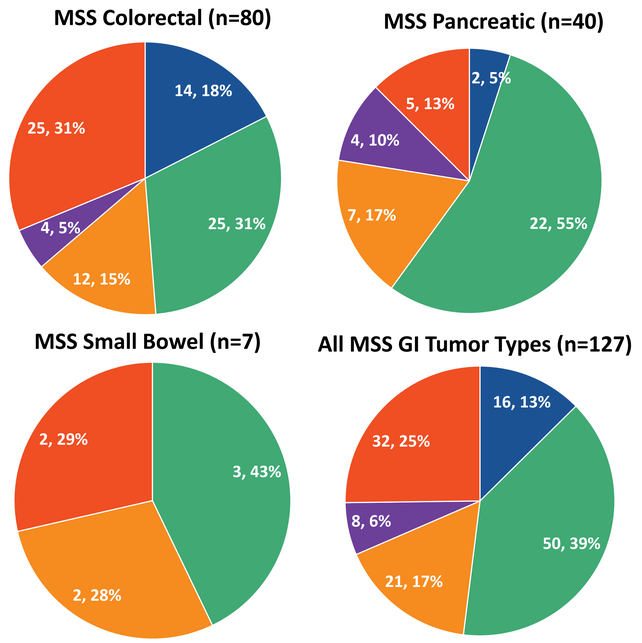
Figure 1. PD-L1 IHC and RNA-Seq workflow

PD-L1 Concordance



- 76% (n=97) of MSS GI tumors were PD-L1 IHC negative (TPS < 1%), with corresponding low/very low PD-L1 RNA-Seq expression values for 63/97 (65%). **34/97 (35%) of the PD-L1 IHC negative tumors had discordant high/very high expression for PD-L1 by RNA-Seq. 24/34 (71%) of these scores were explained by the presence of immune cells not captured by the IHC TPS scoring method.**
- 24% (n=30) of MSS GI tumors were PD-L1 IHC positive (TPS ≥ 1%), but only 3 (2%) of these cases were TPS ≥ 50%.

Immune Subsets by GI Tumor Type



Myeloid Suppressed
Myeloid suppression, defined as overexpression of CD163 or CD68 by RNA-Seq, was the most common immune type across all MSS GI tumors, and was more common in in pancreatic (55%) versus colorectal (31%) tumors.

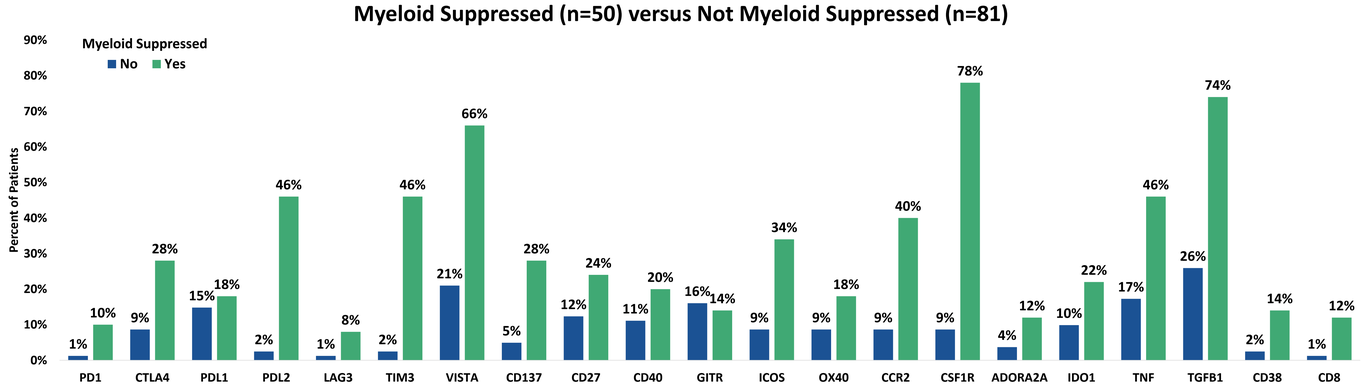
PD-L1 Positive, Inflamed
Overall, very few GI tumors overexpress PD-L1 and have evidence of moderate to high CD8 TILs by RNA-Seq (6%)

PD-L1 Negative, Inflamed
Tumors with evidence of CD8 TILs but no/low expression of PD-L1 by RNA-Seq are more than twice as common in colorectal (31%) versus pancreatic cancers (13%).

Immune Desert
Tumors with no overexpression of known immunotherapeutic targets in clinical development more than three times as common in colorectal (18%) versus pancreatic (5%) cancer.

Other
Tumors that overexpress ≥ 1 other tested immune markers

Immunomodulatory Immunotherapeutic Targets in Myeloid Suppressed GI Tumors



- All myeloid suppressed tumors overexpressed at least one myeloid-related immunotherapeutic target with therapies in clinical development including CCR2, CSF1R, or TGFB1. The majority of these 50 cases (n=37; 74%) also over expressed VISTA, TIM3, or both, indicating that over expression of these two checkpoint blockade receptors are strong indicators of myeloid suppression. PD-L2 was also frequently overexpressed in myeloid suppressed tumors (n=23; 46%).
- Patients with myeloid suppressed GI tumors may also be more likely to benefit from drugs in clinical development that target alternative checkpoint blockade receptors.
- Overall, very few MSS GI tumors overexpressed CD8. Tumors that were not myeloid suppressed however, (38/81; 47%) more commonly had low or very low expression of CD8 and can aptly be called cold tumors.

Marker	Therapies in Clinical Development	Not Myeloid Suppressed	Myeloid Suppressed
ADORA2A	AZD4635; CPI-444; NIR178; PBF-509	24	43
CCR2	BMS-813160	24	29
CD137	Urelumab; Utomilumab; PRS-343	9	18
CD27	Varlilumab	1	3
CD38	Daratumumab; Isatuximab	2	4
CD40	Selicrelumab; ABBV-428; ABBV-927; ADC-1013; APX005M; CDX-1140; SEA-CD40	12	13
CD8	Etirinotecan Pegol; ALKS 4230; ALT-803; NKTR-214	48	12
CSF1R	Cabiralizumab; Emactuzumab; AMG 820; ARRY-382; BLZ945; JNJ-40346527; LY3022855; PD-0360324; SNDX-6352	14	45
CTLA4	Ipilimumab; Tremelimumab; AGEN1884; BMS-986218; BMS-986249; MK-1308; REGN4659; XmAb20717	10	17
GITR	BMS-986156; GWN 323; INCAGN01876; MK-4166; OMP-336B11; TRX518	25	15
ICOS	BMS-986226; GSK3359609; JTX-2011	13	18
IDO1	Epacadostat; Indoximod; BMS-986205; KHK2455; LY3381916; MK-7162; NLG802	19	13
LAG3	Relatlimab; BI 754111; BMS-986213; FS118; INCAGN02385; LAG525; MGD013; MK-4280; REGN3767; Sym022; TSR-033	7	7
OX40	ABBV-368; BMS-986178; GSK3174998; INCAGN01949; MEDI0562; PF-04518600	14	13
PD-1	Cemiplimab; Nivolumab; Pembrolizumab; ABBV-181; AGEN2034; BGB-A317; BI 754091; BMS-986213; JNJ-63723283; MEDI0680; MGA012; MGD013; PDR001; PF-06801591; Sym021; TSR-042; XmAb20717	20	13
PD-L1	Atezolizumab; Avelumab; Durvalumab; CA-170; CX-072; FAZ053; FS118; KN035; LY3300054; M7824	20	13
TGFB1	Fresolimumab; Galunisertib; M7824; NIS793; SAR-439459	30	41
TIM3	BMS-986258; LY3321367; MBG453; Sym023; TSR-022	3	30
TNF	Certolizumab; Lenalidomide; Thalidomide	4	2
VISTA	CA-170	23	39

Conclusion

Using a more sophisticated approach to evaluating the tumor microenvironment in GI tumors 3 major groups including PD-L1 positive, myeloid suppression, and immune deserts can be identified that has major implications for the application of precision immunotherapy for this patient population.