Identification of therapy options for Rare and Resistant Gastrointestinal Stromal Tumors (GIST)

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Abstract (No. 10539)

Background: GISTs are predominantly defined by KIT/PDGFRA mutations which are targetable with a range of kinase inhibitors, however the majority become TKI-resistant (TKI-R). Double (KIT/PDGFRA) wildtype (D-WT) GISTs represent a rare subset of GIST patients in need of treatment options. We investigated a commercial database of theranostic biomarkers for the identification of novel therapy options for GIST.
Methods: 217 GIST cases were evaluated for D-WT and TKI-R. A multiplatform approach of biomarker testing was used and included a combination of sequencing (NGS, Sanger), protein expression (IHC) and gene amplification (ISH).
Results: D-WT (n=15) and TKI-R (n= 23) (including 7 with resistance mutations in the absence of a primary, activating KIT mutation and 4 PDGFRA D842V) were studied for additional targetable alterations. IHC and ISH tests revealed no overexpression or amplification in cMET, EGFR, or HER2. PTEN was intact (positive expression) in the majority of GISTs (92.9% (13/14) D-WT; 100% (19/19) TKI-R). Mutational screening revealed variants in 6/47 genes (excluding cKIT and PDGFRA), most of which are potentially targetable with therapies currently available, or in clinical trials: PIK3CA, ABL, cMET, JAK3, RB1, and VHL. ABL and JAK3 mutations were exclusively found in the TKI-R subgroup. PD-1 positive tumor infiltrating lymphocytes were found in 33% (1/3 D-WT) and 60% (3/5 TKI-R), while PD-L1 tumor expression was found in 67% (2/3 D-WT) and 40% (2/5 TKI-R). Although chemotherapy has historically elicited poor responses in GIST (non-selected patient trials), we observed a high frequency of low expression of predictive markers for gemcitabine (RRM1) and paclitaxel (TUBB3) (77%, 90%; 57%, 73% for D-WT and TKI-R, respectively) and high frequency of TOPO1 overexpression for irinotecan (57%, 32% in D-WT and TKI-R, respectively) which were recently shown to be cytotoxic in TKI-R GIST cell lines (Boichuk, 2014).
Conclusions: A multiplatform approach of theranostic biomarkers identified non-cKIT/PDGFRA therapy options for rare and resistant GIST. Opportunities for investigating new targetable agents and potentially re-visiting cytotoxics with biomarker guidance in these subpopulations are warranted.

Background

- Prior to the identification of the molecular drivers, cKIT and PDGFRA, in GIST, clinical management was similar to other soft tissue sarcomas, which included conventional chemotherapies such as doxorubicin.
- Standard treatment for GIST now includes a repertoire of small-molecule inhibitors including imatinib, sunitinib and regorafenib. As with other targeted approaches, the acquisition of resistance mutations inevitably emerge, and novel therapy approaches are needed for patients who have stopped responding to TKIs.
- In addition, treatment standards for the GIST population lacking cKIT or PDGFRA activating mutations (10-15% of GIST patients) are also needed.
- Interestingly, a recent study[3] demonstrated the surprising sensitivity of GIST cell lines and TKI-R GIST patient-derived xenograft models to non-targeted FDA-approved, chemotherapeutic agents.

Methods

** An additional 5 patients have been identified since the submission of the abstract and included in this analysis*

Two hundred seventeen GIST cases referred to Caris Life Sciences from 2009 – 2015 were evaluated; diagnoses were collected from referring physicians and classified at intake based on pathology and clinical history. Specific testing was performed per physician request and included a combination of sequencing (next-generation sequencing [NGS, 47 genes, hot spot] or Sanger), protein expression (immunohistochemistry or IHC) and gene amplification (CISH or FISH).

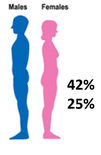
Patients included in this study were those exhibiting wildtype cKIT and PDGFRA genotypes (D-WT) and cKIT/PDGFRA variants associated with resistance to TKI therapy.

Results

A

	D-WT (n=19)	TKI-R (n=24)
Esophagus	(1/19) 5%	(0/24) 0%
Stomach	(6/19) 32%	(6/24) 25%
Small Intestine	(6/19) 32%	(6/24) 25%
Colorectum	(1/19) 5%	(1/24) 4%
Other (abdominal soft tissues, peritoneum, GI Tract, nos)	(5/19) 26%	(11/24) 46%

B



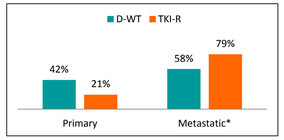
D-WT: 58% 42%
TKI-R: 75% 25%

Mean Age (years): 57 (D-WT), 62 (TKI-R)

C

	cKIT	PDGFRA
Secondary Mutation Present	11 (554_K558del); 17 (Y823D)	WT
	11 (557_K558del); 13(V654A)	WT
	9 (502_Y503dup); 17 (D820E)	WT
	11 (V560D); 13 (V654A)	WT
	11 (V560D); 17 (D820G)	WT
	11 (557_V559delinsF); 13 (V654A)	WT
	11 (L576P); 17 (D820V/D816N)	WT
	11 (554_V560delinsVG); 17 (R815_K818delinsIE)	WT
	11 (552_k558del); 17 (Y823D)	WT
	11 (P551_V555del); 13 (V654A)	WT
	11 (W557R); 13 (H650dup)	WT
	11 (572_P573dup); 17 (D820Y)	WT
	11(551_V555del); 17 (N822K)	WT
Resistance Mutation in Absence of Primary	17 (N822Y)	WT
	17 (N822K)	WT
	17 (N822K)	WT
	17 (N822K)	WT
	17 (Y823D)	WT
	17 (Y823D)	WT
	17 (D820G)	WT
PDGFRA Resistance Mutation	WT	18 (D842V)
	WT	18 (D842V)
	WT	18 (D842V)
	WT	18 (D842V)

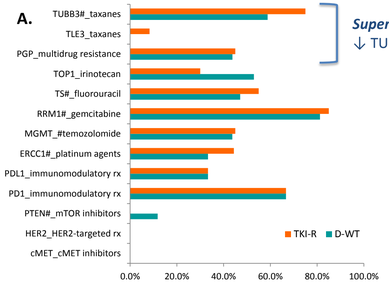
Figure 1A-1C. Primary Tumor Attributes (e.g. site location, genotypes) and Patient Demographics. 1A. Primary tumor sites; most frequent sites were stomach/small intestine for D-WT and gastrointestinal tract, nos for TKI-R. 1B. Male gender and higher mean age associated with TKI-R subgroup. 1C. cKIT/PDGFRA genotypes for TKI-R subgroup.



*Metastatic sites included, for D-WT: abdomen (n=2), connective & soft tissues, chest wall, pelvis, liver (n=2), mesentery, adnexa, colon (n=2) and for TKI-R: abdomen (n=8), pelvis, connective & soft tissue, liver (n=3), omentum, pelvis (n=5), pancreas.

Figure 2. Percent of profiling performed on primary or metastatic tumor specimens. Metastatic sites used for profiling are listed.

Results, continued



Super Selection for Taxane Sensitivity:
↓ TUBB3 + ↓ PGP + ↑ TLE3: 7% (2/28)

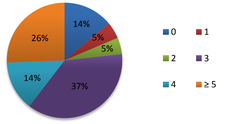


Figure 3A-3B. Protein Biomarkers and Therapy Identification by IHC. 3A. % positivity is shown, unless indicated by #, which are low/negative frequencies (low expression associates with favorable response). Select biomarkers are associated with responses to cytotoxic agents, which were shown to have anti-tumor effects on TKI-R GIST patient-derived xenografts[3]. Immunomodulatory therapies may be of interest as well, based on presence of PDL1 expression in tumor cells and presence of PD1+ TILs. The 2 GIST patients with loss of PTEN expression had normal PIK3CA/AKT genotype. 3B. Predictive biomarker assay by IHC identifies at least one therapy option in 86% of D-WT and TKI-R GIST patients.

**** Note- Changes in gene copy number, assayed by *in situ* hybridization methods, were not detected in cMET (0/14, 0/9) or HER2 (0/16, 0/12) in either subgroup.**

	ABL	ATM	cMET	JAK3	PIK3CA	RB1	RET	TP53	VHL
D-WT	0% (0/19)	5% (1/19)	5% (1/19)	0% (0/19)	5% (1/19)	11% (2/19)	5% (1/19)	5% (1/19)	0% (0/17)
TKI-R	7% (1/14)	0% (0/14)	0% (0/14)	21% (3/14)	7% (1/15)	0% (0/12)	0% (0/14)	0% (0/14)	8% (1/13)

Variants were not detected in the following genes, in either subgroup: AKT1, ALK, APC, BRAF, BRCA1, BRCA2, CDH1, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, HNF1A, HRAS, IDH1, JAK2, KDR, KRAS, MLH1, NOTCH1, NPM1, NRAS, PTEN, PTPN11, SMAD4, SMARCB1, SMO, STK11

Table 2. Variants detected by Next Generation Sequencing for D-WT and TKI-R GIST subgroups. PIK3CA is the only gene, out of the 47 tested, for which variants were detected in both subgroups. Variants in RB1 were detected in two patients in the D-WT subgroup, and JAK3 in three patients in the TKI-R subgroup. A minority of variants detected can be matched to therapies approved for other solid tumors, or therapies under investigation in clinical trials.

	Patient	cKIT	PDGFRA	Additional Gene Alterations and Potential Therapeutic Targets			
				Gene	Variant	Variant Classification/ Functional Significance	Therapeutic Approach
D-WT	1	WT	WT	ATM	D1815fs	Pathogenic, inactivating mutation	DNA-damaging agents, e.g. platinum agents, PARP inhibitors
				RET	Y791F	VUS, likely passenger role	n/a
	2	WT	WT	cMET	T1010I	VUS, weak oncogenic potential	n/a
	3	WT	WT	PIK3CA	E545A	Pathogenic, increased catalytic activity	PIK3CA-AKT-mTOR pathway inhibitors
	4	WT	WT	RB1	R661W	Pathogenic; Partial inactivation of Rb protein	n/a
	5	WT	WT	RB1	R661W		
	6	WT	WT	TP53	C277Y	Presumed Pathogenic	n/a
TKI-R	1	11(P551_V555del); 17 (N822K)	WT	VHL	E160Q	VUS, likely passenger role	n/a
	2	11(V560D); 17 (D820G)	WT	PIK3CA	H1047R	Pathogenic, increased catalytic activity	PIK3CA-AKT-mTOR pathway inhibitors
	3	17 (D820G)	WT	JAK3	V722I	VUS, gain of function variants, activating alleles in AMKL & AML	JAK3 inhibitors being tested for auto-immune diseases, unknown role in cancer
	4	WT	18 (D842V)	JAK3	V722I		
	5	WT	18 (D842V)	JAK3	V718L		
	6	WT	18 (D842V)	ABL1	P408S	VUS	n/a

Table 3. Clinical Implications of variants detected by Next-Generation Sequencing. Therapeutic targets identified by NGS are infrequent events. In D-WT GIST, pathogenic mutations in ATM, PIK3CA, RB1 and TP53 were detected, for which, only PIK3CA and ATM are considered targetable. The most frequently mutated gene in TKI-R GIST, JAK3, has unknown clinical implications in these patients.

Conclusions

- A subgroup of GIST patients, including those that lack activating mutations in cKIT/PDGFRA and those harboring cKIT/PDGFRA resistance mutations, are in great need of therapy options outside of the standard of care
- Preclinical data[3] and predictive biomarker expression distribution presented here, supports "re-visiting" chemotherapy options in a selected population of GIST patients
- IHC identified at least 1 therapy option (chemotherapy and/or targeted therapies not considered standard of care for GIST) in 86% of rare (D-WT) and resistant (TKI-R) GIST.
- Variants detected by NGS offer limited value in identification of targetable alterations. Of the 43 patients included in this study, 3 patients exhibited variants that can be targeted (PIK3CA, ATM).

References

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3. Boichuk, S., A. Duensing, et al. (2014). "Unbiased Compound Screening Identifies Unexpected Drug Sensitivities and Novel Treatment Options for Gastrointestinal Stromal Tumors." Cancer Res 74(4): 1200-1213..

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