Molecularly-guided therapeutic options beyond TKIs for Gastrointestinal Stromal Tumors (GIST)

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Abstract

Background: GISTs are characterized by KIT/PDGFRA mutations. A range of multi-targeted tyrosine kinase inhibitors (TKIs) are available for treatment, however, resistance mechanisms inevitably emerge. Recent data (Boichuk, et al 2014) suggests the potential efficacy of various cytotoxic therapies that were identified as being able to effectively kill TKI-responsive and -resistant GIST cells. We sought to investigate the theranostic markers associated with non-TKI therapy options for their potential role in treatment of GIST.

Methods: 147 GIST cases were evaluated. A multiplatform approach of biomarker testing was used and included a combination of sequencing (NGS, Sanger), protein expression (IHC) and gene amplification (ISH).

Results: Multidrug resistance phenotype was found in 52-68% (PGP, MRP1). Tubulin-binding agents (taxanes, vinca alkaloids) may be of potential use due to the high frequency of low TUBB3 expression (72% or 39/54). Anthracyclines and topoisomerase inhibitors may be of potential benefit in 1/3 of patients based on expression of TOPO2A (32% or 32/99) and TOPO1 (34% or 37/110). Cytotoxic agents used in non-GIST solid tumors, may also be considered, based on high frequency of low expression of MGMT (47% or 57/122), TS (70% or 76/109) and RRM1 (79% or 88/111). PTEN was intact (positive expression) in the majority of GIST (87%). Nine patients were examined for PD1/PDL1: 56% exhibited positive tumor infiltrating lymphocytes and 33% exhibited PDL1 tumor expression. Only one amplification event was observed: cMET (0/53), HER2 (0/69), EGFR (0/16), PIK3CA (0/1) and TOP2A (1/11). Mutational screening using a hot spot cancer panel (and Sanger sequencing for some genes) resulted in the detection of variants in only 10 genes, excluding KIT (97/132) and PDGFRA (5/55). Variants were detected in the following genes, in decreasing order of frequency: RB1, APC and JAK3 (2/55; 2/55; 2/57), PIK3CA (2/69) and ABL1, cMET, EGFR, KDR, VHL and BRAF (1/55, 1/57, 1/57, 1/57, 1/52, 1/78).

Conclusions: A multi-platform approach of theranostic biomarkers identified therapies beyond TKIs for GIST. Various cytotoxics and non-KIT/PDGFRA targeted therapies were identified based on protein expression or gene variations.

*Data is updated to include an additional 67 GIST patients

Background

Prior to the identification of the molecular drivers, cKIT and PDGFRA, in GIST, clinical management of GIST was similar to other soft tissue sarcomas, which included surgery and conventional chemotherapies such as doxorubicin. Standard treatment for GIST now includes a repertoire of small-molecule tyrosine kinase inhibitors (TKIs), including imatinib, sunitinib and regorafenib. As with other targeted approaches, the acquisition of resistance mutations inevitably emerge, and novel approaches are needed for patients who have stopped responding to TKIs. Further, treatment standards for the GIST population lacking cKIT or PDGFRA activating mutations (10-13% of GIST patients) are also needed.

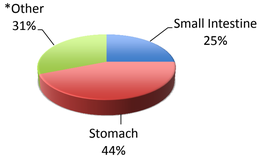
Interestingly, a recent study (Boichuk, et al. 2014, Cancer Res) demonstrates the surprising sensitivity of GIST cell lines and patient-derived GIST xenograft models to non-targeted FDA-approved, chemotherapeutic agents. Here, we explore the non-cKIT and non-PDGFRA aberrations that occur in GIST tumors to uncover potential treatment strategies that include conventional chemotherapy.

Methods

Two hundred fourteen GIST cases referred to Caris Life Sciences from 2009 through 2014 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] or Sanger), protein expression (immunohistochemistry) and gene amplification (CISH or FISH).

Results

Tumor Attributes and Patient Demographics



*other includes patients with confirmed history of GIST, with tumor sites in abdominal soft tissues, peritoneum, retroperitoneum, colon or rectum, esophagus or unknown primary tumor site

Figure 1. Primary Tumor Location. 214 GIST were studied and grouped according to primary tumor site location.

Specimen Site	% (n)
Mesentery, Omentum, Peritoneum, Retroperitoneum	28% (31/109)
Liver	20% (22/109)
Other (bone, chest wall, kidney, mediastinum, spleen, vulva, etc.)	17% (18/109)
Abdomen, NOS	15% (16/109)
Pelvis, NOS	13% (14/109)
Connective tissues, soft tissues	9% (10/109)
Colon	5% (5/109)
Pancreas	3% (3/109)

Table 1. Specimen site for profiling. 51% of specimens profiled were from sites other than the primary tumor site listed, suggestive of metastatic (local and distant) disease.

Patient Demographics: 54% male; 46% female, mean age of 61

Results (contd.)

Boichuk, S. et al. (Cancer Res 74:1200-1213) explored the sensitivity of GIST cells to various FDA-approved chemotherapeutic agents by performing a compound screen using the NCI/NIH Approved Oncology Drugs Set II. Using a pre-defined drug response score, they identified a number of chemotherapeutic agents that had high antitumor activity. We assessed the frequency distributions of GIST patients' protein expression and gene copy number data that associate with several chemotherapies. Agents highlighted in green below were shown to have antitumor effects on GIST cells in Boichuk's study.

Predictive Biomarker (n/total)	% in favor of Response to Rx (based on mechanism)	Therapy	Drug Class
RRM1_low (127/166)	76.50%	gemcitabine	DNA synthesis inhibitor
TUBB3_low (45/62))/ TLE3 (6/80)	72.60%/7.50%	paclitaxel, vinorelbine	Microtubule poison
TS_low (119/165)	72.10%	pemetrexed	Antimetabolite
PD-1 (10/16)/PD-L1 (5/16)	62.50%/31.30%	*nivolumab	Immunomodulatory agent
TOPO1 (77/166)	46.40%	topotecan	Topoisomerase inhibitor
ERCC1_low (49/110)	45.0%	cisplatin	Crosslinking agent
MGMT_low (72/175)	41.10%	temozolomide	Alkylating agent
TOP2A (44/154)/TOP2A FISH(1/11)	28.60%/9.0%	doxorubicin	Topoisomerase inhibitor
EGFR (3/11)	27.3%	*cetuximab	monoclonal antibody
SPARC (69/347)	19.90%	*nab-paclitaxel	Microtubule poison
PTEN_low (32/178)	18.00%	everolimus	Kinase Inhibitor
PR (28/173)	16.20%	anti-hormonal therapy	others
Androgen Receptor (18/172)	10.5%	anti-hormonal therapy	others
EGFR FISH (1/38)	2.6%	erlotinib	Kinase Inhibitor
Estrogen Receptor (4/173)	2.3%	anti-hormonal therapy	others
cMET (1/78)	1.3%	*tivantinib	Kinase Inhibitor
ALK FISH (0/8), cMET ISH (0/57), HER2 (0/173), HER2 ISH (0/79)	0.0%	*crizotinib, *tivantinib, *trastuzumab	Kinase Inhibitor, monoclonal antibody

*drugs were not included in the compound library

Table 2. Frequency distribution of protein and gene copy number changes. All biomarkers above are tested by immunohistochemistry (protein levels), unless indicated by "ISH" (gene copy status by in situ hybridization). Percent frequencies represent data collected from CMI database; highlighted rows correspond to drugs that effectively inhibit GIST cells in Boichuk's study.

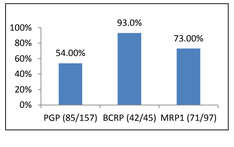


Figure 2. Multidrug Resistance (MDR) Phenotype – majority of GIST patients exhibit overexpression of ABC transporters which are drug efflux pumps.

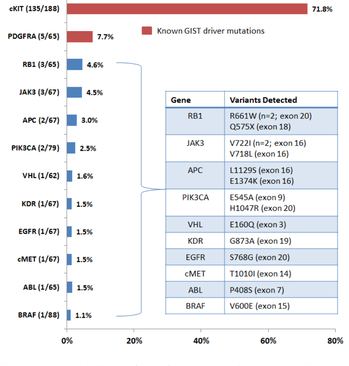


Figure 3. Mutational analysis in up to 188 GIST patients. Data demonstrates that beyond cKIT and PDGFRA, there is limited success at identifying a targetable gene through sequencing platforms.

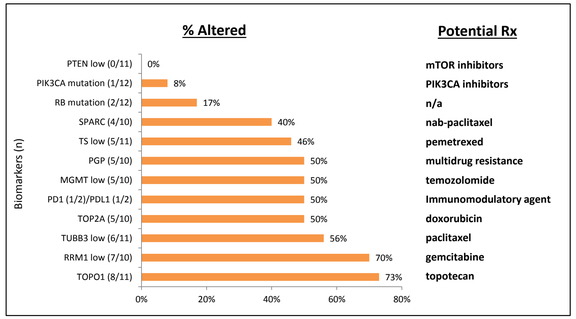


Figure 4. Notable biomarker alterations in cKIT and PDGFRA wildtype GIST patients. Multiplatform profiling including IHC, ISH and NGS platforms revealed several potential therapeutic options based on expression status of multiple predictive biomarkers. Importantly, NGS identified only 2 alterations and ISH did not identify any alterations. Data suggests potential therapeutic options based on protein expression status for cKIT/PDGFRA wildtype GIST patients.

Conclusions

- A multiplatform approach of identifying potential therapeutic options for GIST patients who become resistant to TKI therapy or are cKIT/PDGFRA wildtype may yield therapeutic options beyond tyrosine kinase inhibitors.
- Our data demonstrate GIST patients exhibit high frequency of low RRM1, low TUBB3, low TS and high TOP2A protein expression. These frequencies suggest the potential utility of cytotoxic agents that include DNA synthesis inhibitors, microtubule poisons, antimetabolites and topoisomerase inhibitors.
- GIST patients frequently exhibit high levels of drug efflux pumps, demonstrating the potential role for multidrug resistance, which lends support for the added benefit of identifying treatment options through molecular profiling.
- Mutational platforms offer limited value in detecting targetable genes outside of cKIT and PDGFRA. Non-cKIT/PDGFRA targetable mutants are rare events (e.g. BRAF V600E)
- Protein expression offers the most value for cKIT and PDGFRA wildtype patients (10-13% of GIST population), identifying multiple ,potential treatment choices based on expression status of predictive biomarkers (TOPO1, TUBB3, etc.)

References

1. 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.