Molecular Profiling in Gastric Cancer: Examining Potential Targets for Chemotherapy

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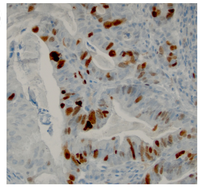
Abstract

Background: Current NCCN guidelines recommend perioperative epirubicin (E), cisplatin (C), and 5-fluorouracil (F) along with other triple agent derivations as first line therapeutic approaches for operable gastric adenocarcinoma (GC). In this study, we utilized molecular profiling to evaluate expression of chemotherapy targeted biomarkers associated with ECF therapy and other first line cytotoxic regimens for gastric cancer.
Methods: Surgically obtained gastric cancer specimens were analyzed by tumor profiling(Caris Life Sciences, Phoenix). Immunohistochemistry for TOP2A, TS, ERCC1, PGP, and TOPO1 expression were analyzed on samples submitted from 2009-2012 for mutually exclusive or simultaneous expression.
Results: A total of 230 GC specimens were analyzed. The median age of patients was 61 (IQR: 50-72) years with the majority being male (n= 139, 60%). IHC actionable targets included: 60% (n=138) high TOP2A, 55% (n=127) negative ERCC1, and 63% (n=145) negative TS, indicating potential benefit from E, C and F respectively. When analyzing for simultaneous expression profiles of the three biomarkers, only 24 % (n=55) of patients had gene expression levels that suggested sensitivity to all three agents (ECF). Moreover, biomarker results of 6.5% (n=15) of patients demonstrated a potential complete lack of sensitivity to first line ECF therapy. Overall, 61% (n=140) of patients had molecular profiles that indicated sensitivity to two or more agents.
Conclusions: Biomarker analysis of GC suggests that there is potential for TOP2A, TS and ERCC1 to define patients who have the greatest likelihood of deriving benefit from ECF therapy. 93.5% of patients had the biomarker profile that predicted sensitivity to at least one of these agents. Prospective controlled studies are required to validate the role of TOP2A, TS and ERCC1 in routine management of GC patients.

Background

- Gastric cancer (GC) continues to carry a grave prognosis, with 5 year overall survival rates of less than 25%
- Aggressive nature of GC makes selecting the optimal systemic chemotherapy regimen critical when attempting to prolong survival.
- Current NCCN guidelines list 16 different first line systemic therapy regimens for treating operable or locally advanced disease
- Not all patients will respond to initial treatment regimens or a subgroup of patients will eventually progress on a therapy that was once effective
- Emergence of molecular profiling has enabled clinicians to measure the activity of potential genetic targets in tumors for which systemic therapy agents are already available
- **Study Objective: To evaluate expression of chemotherapy targeted biomarkers associated with epirubicin (E), cisplatin (C), and 5-fluorouracil (F) therapy and other first line cytotoxic regimens for gastric cancer using molecular profiling**

A) 

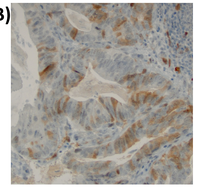
B) 

Figure 1: A) Positive protein expression of TOP2A (antibody clone 3F6, nuclear staining) and B) TS (antibody clone TS 106, nuclear and/or cytoplasmic staining) measured by IHC on a GC specimen 20x).

Methods

- Gastric cancer tissue samples were submitted to Caris Life Sciences (Phoenix, AZ) for tumor profiling analysis aimed to provide theranostic information. Retrospective biomarker analysis was performed on 5 different proteins on 230 samples submitted from 2009-2012.
- Primary antibodies used for protein expression detection were TOP2A (3F6), PGP (C494), TOPO1 (1D6), ERCC1 (8F1) and TS (TS106),
- Avidin-biotin systems and polymer-based, biotin-free systems were used for detection of the antibodies
- The intensity of the staining was scored as 0, 1+, 2+ or 3+, and the percentage of staining cells was scored as 0 to 100%
- IHC thresholds: **high TOP2A**, percentage of staining greater than 10%; **high TOPO1**, staining greater than or equal to 1+, 10%; **negative TS**, staining smaller than or equal to 1+, 25%; **negative PGP**, percentage of staining smaller than 10% and **negative ERCC1**, percentage of staining smaller than or equal to 25%. (Figure 1)

Results

Table 1: Chemotherapy targeted biomarkers and associated cytotoxic agents

Target Biomarker	Associated Agents
TOP2A (+)	Epirubicin
ERCC1 (-)	Carboplatin, Cisplatin, Oxaliplatin
TS (-)	5-FU, Capecitabine
PGP (-)	Paclitaxel, Docetaxel
TOPO1 (+)	Irinotecan

(-), Negative expression; (+), high expression

Table 2: Potential Targets by IHC (Total= 230 patients)

Target Gene	Percent Actionable (n)	Missing
TOP2A (+)	60% (138)	0
ERCC1 (-)	55.2% (127)	0
TS (-)	63% (145)	0
PGP (-)	54.8% (120)	11
TOPO 1 (+)	67.5% (154)	2

(-), Negative expression; (+), high expression

Table 3: Simultaneous expression analysis of biomarkers associated with ECF therapy.

	TOP2A (+)		TOP2A (-)	
	TS (-)	TS (+)	TS (-)	TS (+)
ERCC1 (-)	**23.9% (55)**	**10% (23)**	**14.8% (34)**	6.5% (15)
ERCC1 (+)	**12.2% (28)**	13.9% (32)	12.2% (28)	6.5% (15)

(-), Negative expression; (+), high expression

High TOP2A, negative TS and negative ERCC1 are associated with potential benefit from epirubicin, fluoropyrimidine and platinums, respectively. (Total=230 patients, percent actionable (n)). The bolded are the biomarker combinations with two or more agents indicated as beneficial.

Table 4: Simultaneous expression analysis of TOPO1 and PGP

	TOPO1 (+)	TOPO1 (-)
PGP (-)	31.8% (69)	35.5% (77)
PGP (+)	22.6% (49)	10.1% (22)

(-), Negative expression; (+), high expression

High TOPO1 and negative TS are associated with potential benefit from irinotecan and taxanes, respectively (Total=217)

Results *(continued)*

Table 5: Predicted sensitivity to preferred first line systemic chemotherapy regimens according to NCCN Guidelines for resectable as well as locally advanced gastric cancer (including EGJ adenocarcinoma)

Chemotherapy Regimen	Associated Target Gene(s)	% Predicted Sensitivity
ECF/ Modifications (Epirubicin, Cisplatin/Oxaliplatin, Capecitabine/ Fluorouracil)	TOP2A, ERCC1, TS	At least one agent: 93.5% Two or more agents: 60.8% Three agents: 23.9%
Fluoropyrimidine (Fluorouracil/ Capecitabine) and Cisplatin/ Oxaliplatin	TS, ERCC1	At least one agent: 79.6% Two agents: 38.7%
DCF/ Modifications (Docetaxel, Carboplatin/Oxaliplatin, Fluorouracil)	PGP, ERCC1, TS	At least one agent: 88.7% Two or more agents: 60.4% Three agents: 22.6%
Fluorouracil and Irinotecan	TS, TOPO1	At least one agent: 87.8% Two agents: 42.2%
Paclitaxel/ Docetaxel with ciplatin/ carboplatin	PGP, ERCC1	At least one agent: 76.7% Two agents: 33.3%
Docetaxel and irinotecan	PGP, TOPO1	At least one agent: 89.9% Two agents: 31.7%
Fluoropyrimidine Alone (Fluorouracil/Capecitabine)	TS	63%
Docetaxel or Paclitaxel Alone	PGP	54.8%

Conclusions

- **Retrospective biomarker analysis of 230 gastric cancer patient samples show that approximately one of four patients possess a molecular profile that would predict uniform sensitivity to ECF therapy**
- **93.5% of patients had a biomarker profile that predicted sensitivity to at least one chemotherapy agent, while 6.5% of patients demonstrated a potential complete lack of sensitivity to first line ECF therapy.**
- **Molecular profiling is emerging as a diagnostic adjunct and identification of a chemo-resistant biomarker profile could potentially suggest an alternative treatment plan.**
- **Prospective controlled studies are needed to validate the role of these biomarkers in identifying effective cytotoxic agents for gastric cancer**

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