Profiling of 1,250 neuroendocrine tumors identifies multiple potential drug targets

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Abstract #214

Identification of new drug targets may extend treatment options for neuroendocrine tumors (NET), regardless of histologic classification or primary organ site. **Methods**: 1,250 cases of infradiaphragmatic neuroendocrine tumors (all grades and sites) were identified among >50,000 cases profiled in a CLIA-certified laboratory. Biomarker profiling utilized multiple platforms: gene sequencing (next generation sequencing [NGS], Sanger or pyrosequencing), gene copy number by *in-situ* hybridization, and protein expression by immunohistochemistry (IHC). The results are shown relative to the total number of tests performed. **Results**: Overall, drug therapy-relevant alterations were identified in 1130 of 1250 (90%) of cases. Low or absent (0 or 1+ by IHC) expression ofMGMT a biomarker of sensitivity to alkylating agents, was found in 130/219 pancreatic cases (59%), and in 450/991 (45%) of non-pancreatic NET. Low or absent (0 or 1+ by IHC) expression of RRM1, a biomarker of gemcitabine sensitivity, was found in 813/1100 of NET (74%) and low or absent thymidine synthase, TS, a biomarker of fluoropyrimidine sensitivity, was shown for 793/1096 (72%) of NET by IHC. Sequencing of tumors showed oncogenic mutations in BRAF (4/369 (V600E in 3 and G596R in 1), CTNNB1 (2/150), KIT (3/281), EGFR (1/178), FGFR2 (1/150), GNAS (1/150), HRAS (2/150), PIK3CA (6/343), RB (2/150) VHL (1/150), KRAS (10/125), NRAS (2/274), and APC (2/150) and amplifications of EGFR (46/686) and MET (4/236). Ki67 status and correlation between the site of origin and biomarkers will be presented. Therapies guided by mechanism-based biomarkers produced durable responses in documented cases: partial response (PR) >1 year to imatinib in a patient with KIT-mutant metastatic NET, and in cases of $MGMT^{low}/TS^{low}$ treated with streptozocine or temizolomide plus fluoropyrimidine chemotherapy, thus supporting the clinical relevance of target profiling in NET. **Conclusions**: Comprehensive multiplatform profiling of a large series (n=1250) of NET, despite low frequency of individual biomarkers, identified clinically relevant targets in the majority of patients. Our results provide the basis for future clinical trials to assess the efficacy of biomarker-based therapy for NET.

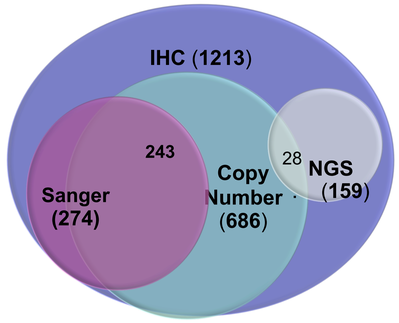
Methods

- All neuroendocrine tumor cases referred to Caris Life Sciences between 2009 thru Sep. 2013 from 50 states and 30 countries 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 (Sanger, NGS or pyrosequencing), protein expression (immunohistochemistry), and/or gene amplification (CISH or FISH).
- Statistical analysis was performed using JMP and the Fisher two tail test was used to report p values.
- MGMT protein expression was low when <35% tumor cells stained intensity ≤ 1+.
- RRM1 protein expression was low when <50% tumor cells stained intensity ≤ 2+.
- TS protein expression was low when <10% tumor cells stained intensity ≤ 3+.

Biomarker panel, Caris Molecular Intelligence™ Profiling

Tests performed:	N pts:
1.IHC- 30 biomarkers	900-1213
2.FISH/CISH- 6 biomarkers	200-686
3.Sanger seq- 6 biomarkers	100-274
4.NGS- 44 gene panel	159

(a range is shown when not all biomarkers were tested for a single case)



Results

Potential chemotherapy biomarkers

Marker	Pancreatic NET	Non-pancreatic	P-value P.NET vs. Non-p	Drug
MGMT low (IHC)	130/219 (59%)	450/991 (45%)	0.0002	Alkylators (temozolamide)
RRM1 low (IHC)	166/191 (87%)	666/910 (73%)	0.0001	Gemcitabine
TS low (IHC)	180/191 (94%)	796/905 (88%)	0.0104	5FU, capecitabine

Potential Drug-amenable genetic alterations

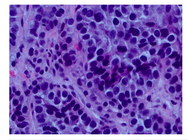
Gene	Mutation	Domain	Frequency	Drug
KIT	L647F V560del D579del V532I	Kinase Membrane Helical	4/281 (1.4%)	Imatinib, sunitinib
BRAF	K601E V600E (3) G596R G469A	Kinase Kinase Kinase	6/369 (1.6%)	Vemurafenib
EGFR	CN increase		46/686 (6.7%)	Erlotinib, cetuximab
EGFR	G719S	Kinase	1/178 (0.6%)	Erlotinib
PI3KCA	H1047R (3) M1043I (2) E542K E110_N114delinsD	Kinase Kinase Helical p85 Binding	7/343 (2.0%)	Buparlisib*
FGFR2	A379T C382R	Membrane	2/150 (1.3%)	Dovitinib*
MET	CN increase		4/236 (1.7%)	Crizotinib
MET	D174N T1010I (2)	Membrane	3/157 (1.9%)	Crizotinib

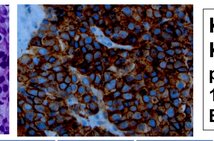
*Investigational drug

28 of 44 genes in the NGS panel tested positive for a mutation in at least one case; HNF1A, SMAD4, VHL mutations were seen only in pancreatic NET; APC, BRAF, KRAS, NRAS, TP53 mutations were seen in both pancreatic and non-pancreatic NET.

Case #1

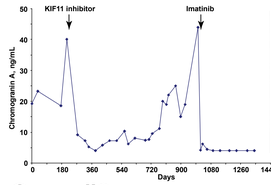
A 48 y.o. male presented in 01/2010 with massive hepatomegaly due to diffuse and nodular metastatic NET of unknown primary. Patient had Hx of testicular cancer in 1988 treated with surgery & RT. He has a maternal relative with leukemia. Conventional chemotherapies failed: 1) Cisplatin+Etoposide; 2) 5FU+Streptozocin; 3) Phase I Eg5 kinesin inhibitor produced PR, progression in 2012. Repeat biopsy sent for Caris Molecular Intelligence ™ profiling, with resulting imatinib treatment, per Caris report:

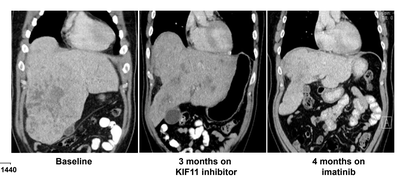




Ki67-20% tumor cells
KIT- 3+ 100% by IHC
p. V560del c. 1679_1681delTTG
Began Imatinib in 10/2012

Agents Associated With CLINICAL BENEFIT	Biomarker	Result	Method	Summary Statement
temozolomide	MGMT	Negative	IHC	Low expression of MGMT has been associated with benefit from temozolomide.
erlotinib, gefitinib	EGFR	Negative	FISH	Although EGFR FISH is negative, patients with adequate levels of PTEN may benefit from EGFR-targeted tyrosine kinase inhibitors.
	PTEN	Above Threshold	IHC	
cetuximab, panitumumab	PTEN	Above Threshold	IHC	Although EGFR FISH is negative, patients with adequate levels of PTEN may benefit from EGFR-targeted antibodies.
	EGFR	Negative	FISH	
imatinib	c-kit	Mutated - Exon 11	Molecular	Presence of c-Kit mutation in exon 11 has been associated with benefit from imatinib.
nab-paclitaxel	SPARC Polyclonal	Above Threshold	IHC	High expression of SPARC has been associated with benefit from nab-Paclitaxel.
	SPARC Monoclonal	Negative	IHC	
irinotecan	TOPO1	Above Threshold	IHC	High expression of TOPO1 has been associated with benefit from irinotecan.



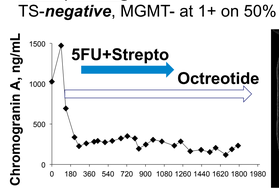


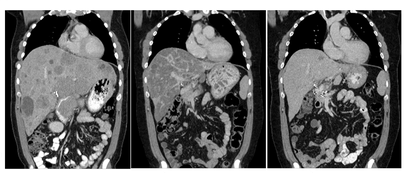
Case #2

A 33 y.o. male presented in 08/2008 with Zollinger-Ellison syndrome: metastatic gastrinoma with multiple peptic ulcers, hepatomegaly and a large mass in the head of pancreas (PNET). with resulting 5FU treatment, per Caris report:

Caris profiling

TS-***negative***, MGMT- at 1+ on 50% of cells. No base mutations.





Conclusions

- **Multi-platform profiling, measuring gene amplification, mutation and/or protein expression identified alterations in 93% of NET; 91% aid in treatment selection**
- **Drug-amenable alterations (amplification or mutation) are found in at least 17% of all NET**
- **Additional biomarkers of chemotherapy sensitivity are worth exploring in a systematic study (5FU, alkylating agents, gemcitabine)**
- **Therapeutic selection based on information provided by a commercially available multi-platform molecular profiling service produced durable responses in select patients**
- **Given the expanding number of potential treatments for this group of relatively indolent tumors, further study and expansion of this panel of markers is warranted.**