Profiling of 1,350 neuroendocrine tumors identifies multiple potential drug targets

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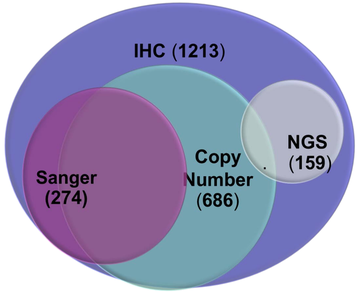
Abstract #4113

Background: Identification of new drug targets may extend treatment options for neuroendocrine tumors (NET) regardless of histologic classification or primary organ site.

Methods: 1,350 cases of neuroendocrine tumors (all grades and sites) were identified among >60,000 cases profiled in a CLIA-certified laboratory. Biomarker profiling was performed on formalin-fixed, paraffin-embedded tumor samples (fresh samples were not needed) and utilized multiple platforms: gene sequencing (next generation sequencing [NGS], Sanger or pyrosequencing), gene copy number (in-situ hybridization), and protein expression (immunohistochemistry (IHC)). The results are shown relative to the total number of tests performed.

Figure 1. Number of cases tested by each technology.



Results: Overall, drug therapy-relevant alterations were identified in 1295 of 1350 (96%) of cases. Low or absent (0 or 1+ by IHC) expression of MGMT a biomarker of sensitivity to alkylating agents, was found in 149/243 pancreatic cases (61%), and in 488/1015 (48%) of non-pancreatic NET. Low or absent (0 or 1+ by IHC) expression of RRM1, a biomarker of gemcitabine sensitivity, was found in 927/1193 of NET (78%) and low or absent thymidine synthase, TS, a biomarker of fluoropyrimidines sensitivity, was shown for 950/1187 (80%) of NET by IHC. Sequencing of tumors showed oncogenic mutations in BRAF (6/446 (V600E in 3, G596R in 2, and K601E in 1), CTNNB1 (3/223), KIT (4/357), EGFR (1/245), FGFR2 (2/224), GNAS (1/224), HRAS (2/192), PIK3CA (10/418), RB1 (4/222) VHL (2/203), KRAS (23/472), NRAS (2/349), and APC (14/224) and amplifications of EGFR (46/688) and MET (4/306. 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 streptozocin or temozolomide plus fluoropyrimidine chemotherapy, thus supporting the clinical relevance of target profiling in NET.

Conclusion: Comprehensive multiplatform profiling of a large series (n=1350) of NET, despite low frequency of individual biomarkers, identified clinically relevant targets in >90% of patients. Given the increasing utilization of chemotherapy for NET, our results provide the basis for future clinical trials to assess the efficacy of biomarker-based therapy for NET.

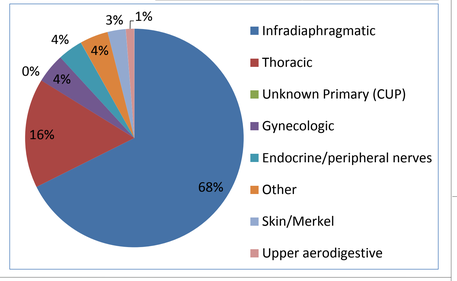
Demographics, Histology

Table 1. Demographic information.

*Metastatic status was not always available

% Female	54
% Male	46
Median Age	60
Age Range	1-99
Metastatic*	762 (56%)

Figure 2. Primary site, shown as percent of total 1350 cases.



Results: Potential drug-amenable genetic alterations

Table 2. Gene mutations of interest, frequency, subtype, and potential therapeutic options are shown for drug-amenable mutations

Gene	Mutation	Domain	Frequency	Subtype	Drug
KIT	L647F V560del D579del V532I	Kinase Membrane Helical	4/357 (1.1%)	2 thoracic 1 infradiaphragmatic 1 Unknown Primary	Imatinib, sunitinib
BRAF	K601E V600E (3) G596R G469A	Kinase Kinase Kinase	6/446 (1.3%)	2 thoracic 3 infradiaphragmatic 1 Unknown Primary	Vemurafenib
EGFR	CN increase		46/686 (6.7%)	21 infradiaphragmatic 13 thoracic 6 Unknown Primary 6 other	Erlotinib, cetuximab
EGFR	G719S	Kinase	1/245 (0.4%)	1 bladder	Erlotinib
PI3KCA	H1047R (3) M1043I (3) E542K , E545K, E110_N114delinsD D1017H	Kinase Kinase Helical p85 Binding	10/418 (2.4%)	3 Unknown Primary 2 infradiaphragmatic 4 other (1x2MT)	Buparlisib
FGFR2	A379T C382R	Transmembrane	2/224 (0.9%)	infradiaphragmatic	Dovitinib
MET	CN increase		4/236 (1.7%)	3 infradiaphragmatic 1 thoracic	Crizotinib
MET	D174N T1010I (2)	Extracellular Cytoplasmic	3/157 (1.9%)	2 infradiaphragmatic 1 Unknown Primary	Crizotinib

CN=copy number

Total 16.4% associated with therapies

Results:

Table 3. Protein biomarkers of chemotherapy sensitivity. Variability of protein expression suggest personalized treatment options for NET.

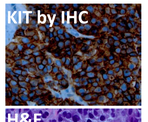
Marker	Pancreatic NET	Non-pancreatic NET	p-value	Drug
MGMT low (IHC)	149/243 (61%)	488/1015 (48%)	0.0002	Alkylating agents (temozolomide)
RRM1 low (IHC)	166/191 (87%)	666/910 (73%)	0.0001	Gemcitabine
TS low (IHC)	180/191 (94%)	796/905 (88%)	0.0104	5FU, capecitabine

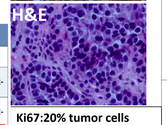
Illustrative cases of CMI-tailored therapy

Case #1

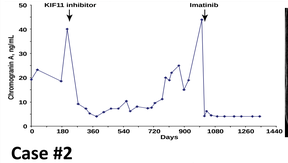
A 48 y.o. male presented in 01/2010 with massive hepatomegaly due to diffuse and nodular metastatic NET of unknown primary. Patient had history of testicular cancer in 1988 treated with surgery & RT. He has a maternal relative with leukemia. Conventional chemotherapies failed: 1) Cisplatin+Etoposide;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

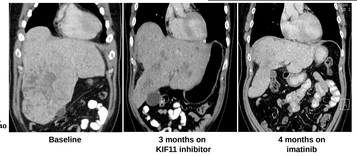
Agents Associated With CLINICAL BENEFIT	Biomarker	Result	Method	Summary Statement
temozolomide	MGMT	Negative	IHC	Low expression of MGMT has been associated with benefit from temozolomide.
erlotinib, gefitinib	EGFR	Negative	FISH	Although EGFR FISH is negative, patients with adequate levels of PTEN may 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.





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



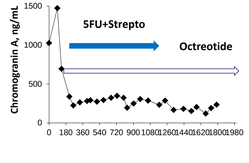


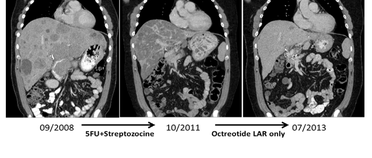
Case #2

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:

Caris profiling

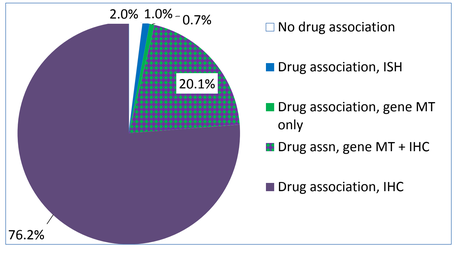
TS-***negative***, MGMT- at 1+ on 50% of cells. No base mutations.





Study Highlights: Potential therapy options

Figure 4. Drug associations using Caris Molecular Intelligence. Cases that were profiled using all 3 technologies were evaluated to identify frequency of molecular aberration found by technology used. More than 96% of cases had a molecular aberration that could be correlated to a potential therapy option, based on protein expression, while **only 1% were identified by gene mutation analysis alone**.



98% of all cases tested had molecular aberrations resulting in therapy recommendations. On average, 8 drugs associated with potential benefit were reported per patient. Of the average 22 total associations per case (either benefit or lack of benefit), an average 10.5 drugs were targeted and 11.5 were conventional therapies.

Conclusions

- Multi-platform profiling, measuring gene amplification, mutation and/or protein expression identified drug-amenable alterations in 96% of NET; gene amplification or mutation alone identified alterations in only 20% of all NET profiled
- 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.

References

Peng L, Schwarz RE. Pancreatic neuroendocrine tumors: signal pathways and targeted therapies. Curr Mol Med. 2013 Mar;13(3):333-9.

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