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Usefulness and Applicability of Next Generation Sequencing in Neuroendocrine Neoplasms

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Abstract

Background: Neuroendocrine neoplasms (NENs) are rare tumors that can arise anywhere in the body and treatment options are limited due to their rarity. Knowledge of their mutational status might allow for tumor agnostic treatments, suggest a familial component or aid in enrollment in clinical trials, especially in the metastatic setting.

Objective: We aimed to evaluate the clinical relevance of results from next generation sequencing (NGS) in NEN patients and determine their applicability to patient management.

Patients and methods: Eligible NEN patients on an institutional, IRB approved protocol, who had NGS as standard of care and were treated in the past 24 months, were included. Tumors were categorized by location and histologic grade. We explored the actual and theoretical eligibility for tumor agnostic treatments and enrollment in clinical trials as available on clinicaltrials.gov.

Results: Between August 2017 and July 2019 a total of 107 patients were eligible. Globally 102 clinical trials included patients with NEN and specific mutations. NGS detected one (1%) case of MSI high and one (1) TRK fusion positive tumor, eligible for checkpoint inhibitor and TRK inhibitor therapy respectively. Moreover, tumor NGS identified 16 (15%) cases of MEN1, 1 (1%) of RET, 2 (2%) of NF1 and 3 (2.8%) of MUTYH, 2 (2%) TSC or TSC2, BRCA in 1 (1%). These patients were appropriately referred to genetic counseling. About 51.5% of patients would in theory be eligible for an investigational treatment based on NGS and global clinical trial availability. Fifty two of 107 patients (48.5%) would not have been eligible for a clinical trial with reasons varying between no mutations (24%), sample failure (8.4%) or nonactionable mutations (15.9%).

Conclusion: NGS can point to clinical trial eligibility and guide genetic counseling and should probably be considered as a standard approach in the evaluation of new metastatic NEN patients.

Keywords: Next Generation Sequencing • Neuroendocrine neoplasms • Clinical trials • Germline testing

Introduction

Neuroendocrine neoplasms (NENs) are rare tumors arising anywhere in the body, most commonly in gastrointestinal tract, pancreas and lungs. Their incidence is rising and an analysis of the Surveillance, Epidemiology and End Results (SEER) database estimated it at 6.98 cases per 100,000 people in the year 2012 [1]. Most NENs seem to be sporadic, but may also arise in the context of inherited genetic syndromes, including multiple endocrine neoplasia (MEN) types 1 and 2, which are associated with mutations in the *MEN1* gene and the *RET* proto-oncogene, respectively, Von Hippel Lindau disease, tuberous sclerosis complex and neurofibromatosis [2-5]. The proper diagnosis and treatment of NENs often involves a multidisciplinary team comprising of pathologists, endocrinologists, radiologists, as well as medical, radiation, and surgical oncologists. Treatments include somatostatin analogues, targeted therapies such as everolimus and sunitinib and peptide receptor radionuclide therapy or a clinical trial. While physicians have more options nowadays, treatments are still limited and there is lack of comparative efficacy data, preventing tailored treatment regimens [6-8].

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Mutational analysis of tumor genes either targeted or as part of next generation sequencing (NGS) platforms, have gained popularity in and out of academia, especially for cancers with well described prognostic or predictive mutations. For example, NGS is used in choosing targeted treatments for *ROS1* or *ALK*-positive lung cancers or *RAS-wild type* colorectal cancers with significant improvements in survival [9-13]. It can also guide treatments for cancers regardless of the primary tumor location (tumor agnostic treatments). For example, larotrectinib, a potent and highly selective tropomyosin receptor kinase (TRK) inhibitor, was approved for treatment in TRK fusion-positive cancers in adults and children regardless of the tumor type or of the age of the patients and the United States, Food and Drug Administration (FDA; MD, USA) approved a second agent, entrectinib for neurotrophic tropomyosin receptor kinase (NTRK) fusion positive cancers and ROS1-positive NSCLC [14,15]. Furthermore, pembrolizumab an immune check point inhibitor was approved for treatment in pediatric and adult patients with any microsatellite instability high (MSI-H) or deficient mismatch repair (dMMR) cancer [16,17].

Obtaining NGS in neuroendocrine neoplasms is not standard of care because there is no known targetable mutation that is exclusive to NENs. However, it is widely used in the academic setting as it can still identify targets for tumor agnostic treatments and suggest familial syndromes. Moreover, in some cases it can allude, but not reliably predict efficacy of a specific treatment, based on laboratory data or commonly described mechanisms of disease [18]. In the tertiary center setting, it can also sometimes affect eligibility of patients for first in-human or tumor agnostic studies that require a specific genetic mutation. The effect of that practice has not been quantified in our experience. Our institution sees a large proportion of metastatic cancer patients for evaluation and treatment, some of which have had their treatments affected by results of NGS. Thus, we sought to describe the mutational landscape of our NEN patients and document the theoretical and actual applicability of NGS.

Materials and Methods

Patient data

This was a retrospective patient study. We perused an institutional review board (IRB) approved registry database of metastatic patients treated or evaluated at our institution in the past 2 years who have had a pathologically confirmed NEN. This included tumors of the gastrointestinal tract and lung, but also pheochromocytoma and adrenocortical cancers. We excluded small cell lung cancer but included other lung neuroendocrine variants; we also excluded Merkel cell tumors. We restricted our search to patients with an attempt at NGS regardless of success. We only included adults over 18 years of age. We extracted the following: patient age and sex, pathology, tumor origin, WHO grading, metastasis on presentation and NGS data. The study was approved by the Ethics committee and institutional IRB and no patient contact was attempted.

NGS data

Immunohistochemistry for mismatch repair protein deficiency and PD-L1/PD-1 expression, as well as for specific mutations (such as p53) were on occasion performed at diagnosis by an institutional panel to clarify histology (for example, in well differentiated high grade tumors) or to justify immunotherapy use. However, all specimens in this study were subsequently analyzed by a commercial panel (FoundationOne, Foundation Medicine, and Cambridge, MA). This comprises of sequencing of DNA obtained from formalin-fixed, paraffin embedded tissue that can detect substitutions, insertion and deletion alterations and copy number alterations in 324 genes and select gene rearrangements. The same panel can also detect genomic signatures including MSI and tumor mutational burden (TMB). Extracted NGS information included tumor mutational burden, PD1 and PD-L1 expression, deleterious mutations as well as variants pointing to a possible syndrome. Variants of unknown significance were excluded.

In the event of a suspicious hereditary syndrome based on tumor mutations (e.g. *BRCA and MEN1*), patients were offered genetic counseling either locally of through their oncologists. Our local option included germline testing with a 73 gene panel (www.invitae.com) from peripheral blood.

Identification of potential clinical trials

We queried clinicaltrials.gov dated July 5th 2019 for all tumor agnostic trials that could have provided options for our patients on that day. To that effect, we utilized the broad terms “solid tumor” and “mutation” as identifiers and included recruiting, not yet recruiting, enrolling and active trials. We excluded suspended, terminated, completed or withdrawn/unknown status and trials focusing mainly on pediatric populations. We also perused the actual eligibility criteria for each trial as documented on the website to roughly determine if neuroendocrine tumor patients would be excluded. We did not contact study administrators or PIs for slots and we did not peruse patient details for actual eligibility, as this was a theoretical exercise.

Results

A total of 107 patients were included in our study. Details are included in Table 1. Mean age was 58.8 years, male to female ratio was 1:1 and most patients (56%) were less than 60 years old. Most common origin was gastrointestinal in 36 of patients (33%) and pancreas in 31 (29%). Most gastrointestinal NENs originated in the small bowel (63.9%) followed by colon (27.8%), rectum (5.6%) and appendix (2.8%). Of the gastroentero-pancreatic and bronchopulmonary tumor patients, 32 (29%) had grade 1, 45 patients had grade 2 (43%) and 15 patients had grade 3 (14%) tumors, as defined by the World Health Organization (WHO).

NGS data

For full details please refer to Table 2. Small bowel tumors did not commonly exhibit mutations (no data in 56.5% of samples), in contrast to pancreatic or high-grade tumors. The most common gastrointestinal mutations were *APC (11.1%), BRAF (8.3%), RB1 (8.3%), TP53 (8.3%), CDKN1B (5.5%)* and *MYC (5.5%)*. The most common mutation in the small bowel tumors was *CDKN1B (8.7%).* We observed that 54.8% of pancreatic NEN patients harbored ≥2 variant mutations, with the most common being *MEN1 (38.7%), DAXX (19.4%), TP53 (13%), PTEN (9.7%), BCOR (9.7%) and ATRX (9.7%).* Higher histologic grade of tumor (when available) was associated with more mutations with 53.2% of grade 1, 77.8% of grade 2 and 100% of grade 3 patients having significant mutations, respectively. The most common genetic mutations in grade 3 NENs in our study were *TP53, PTEN* and *RB1,* which were 40%, 33.3% and 20% respectively. Additionally, we identified *PD-1*≥1% in 4 (3.7%) cases of grade 1, 1 (1%) case of grade 2 and 4 (3.7%) cases of grade 3 and also found *PD-L1*>1% in 4 (3.7%) cases of grade 1, 2 (1.9%) cases of grade 2 and 7 (6.5%) cases of grade 3 NEN (Table 3).

Eligibility for FDA approved therapies and genetic counseling

NGS identified one (1%) *MSI* high/ TMB high patient, 2 (2%) TMB high and 1 (1%) *TRK* fusion positive patient eligible for FDA approved tumor agnostic treatments. Both *MSI* high patients have received immunotherapy with good response, while the *TRK* fusion patient has not progressed on standard

Table 1. Characteristics of the patients included in the study.

Characteristics	N (107)	Percentage
Age (y)		
Mean	58.8	--
<60	60	56.0
≥60	47	44.0
Sex		
M	55	51.4
F	52	48.6
Origin		
GI	36	33.6
Pancreas	31	29.0
Insulinoma	2	
Gastrinoma	1	
Non-functional	28	
Lung	2	1.8
Adrenocortical	3	2.8
Cervix	1	1.0
Ovary	1	1.0
Unknown	33	31.0
WHO Grade		
1	32	29.0
2	45	43.0
3	15	14.0
No report	15	14.0
***PD-L1* expression**		
Yes	12	11.2
No	91	85.0
No report	4	3.7
***PD-1* expression**		
Yes	11	10.3
No	92	86.0
No report	4	3.7
MSI		
High	1	1.0
Intermediate	1	1.0
Low	93	87.0
No report	12	11.2
TMB >17		
yes	2	2.0
no	105	98.0

Table 2. Actionable mutations on patient sample based on results from clinicaltrials.gov.

Genetic mutation	Primary cancer Location									
	Adrenal (2)	Appendix (1)	Cervix (1)	Colon (10)	Small bowel (23)	Lung (2)	Ovary (1)	Pancreas (31)	Rectum (2)	Unknown (33)
Sample failure		1		2	1					1
Failed report					3			1		
None				1	13			2	1	9
ACVR1B										1
AKT2										1
ARID1A					1	1		1		2
ATRX	1							3		
BAP1	1									
BCL2										1
BRAF				3						1
BRCA2										1
CDK4						1				
CDK8										1
CDKN2A/B,1A/B					2		1	8		2
CCND				1		1				
CHEK2					1					
DDR1					1					
EGFR				1						
FLT3										3
ERBB2							1	1		
FBXW7				1				1		
FGFR				2						
FGFR1										1
HRAS					1					
IDH-1								1		
JAK								1		1
KEAP1										1
KRAS							1	2	1	2
MDM2				1		1				1
MET								1		
MLL2						1				1
MLH1								1		
MSH2								1		
MSI								1		
MTOR								1		
MYC				1	1					
NF1								1		1
NTRK1										1
PD-1≥1%				2	1			2		5
PD-L1≥1%	1		1	1				5		4
PIK3							1	1		
PPP2R1A								1		
PTEN				1		1		3		2
RB1				1	1	1			1	5
RET	1									
SETD2								2		
SMARCA4	1									
SMARCB1								1		
SRC				1						
STK11						1				1
TP53				2	1	1	1	4		7
TSC								2		

therapy and thus has not been treated. Furthermore, NGS detected a total of 24 potential germline mutations in 21 patients: 16 (15%) *MEN1*, 2 (2%) *NF1*, 3 (2.8%) *MUTYH*, 2 (2%) *TSC/TSC2*, one *RET* alteration and one deleterious *BRCA* positive tumor specimen. These patients were ultimately offered genetic counseling (Table 4). Almost half (9/21) of patients declined for various reasons, three did because they already carried the clinical diagnosis and two are pending. Germline testing confirmed *MEN1* mutation in a fourth patient and discovered a *CHECK2* mutation in a fifth.

Theoretical eligibility for clinical trials

Our broad search algorithm ran on July 5th, 2019 retrieved 192 studies on clinicaltrials.gov. After careful examination of inclusion and exclusion criteria,

Table 3. Tumor grading and common mutations by next generation sequencing.

	Grade 1				Grade 2				Grade 3 and Poorly diff.			
Locations	**No.**	**Common mutations**	***PD-1* ≥1**	***PD-L1* ≥1**	**No.**	**Common mutations**	***PD-1* ≥1**	***PD-L1* ≥1**	**No.**	**Common mutations**	***PD-1* ≥1**	***PD-L1* ≥1**
Colon	5/10 None (1)	*SRC, APC*	1	0	1/10	*MUTYH, PAX5*	0	0	4/10	*BRAF, APC, TP53, FGF*	1	*1*
Ovary	1/1	*ERBB2, KRAS, PIK3CA, TP53, CDKN2A/B, CHD4, NOTCH3, TOP2A*	0	0								
Pancreas	7/31 None (1)	*MEN1, CDKN1B, KRAS, MET, KDM6A, SF3B1*	1	1	19/31 None (1)	*MEN1, DAXX, TP53, ATRX, SETD2, CDKN1A, TSC*	1	2	5/31	*PTEN, PRKAR1A, CDKN1A, NF1, TP53*	0	2
Rectum	1/2	*None*	0	0					1/2	*APC, KEL, KRAS, RB1*	0	0
Small Bowel	9/23 None (5)	*DNMT3A, TP53, CDKN1B, FAM46C*	0	1	12/23 None (7)	*ARID1A, CHEK2, DDR1, RB1, HRAS*	0	0	2/23 None (1)	*CDKN1B, MYC*	0	0
Unknown	8/33 None (7)	*NTRK1*	2	2	13/33 None (2)	*MEN1, CDKN1B,NF1, RICTOR,FLT3*	0	0	13/23	*TP53, RB1, PTEN, ARID1A, FLT3, APC*	3	2
Adrenal									2/2	*ATRX, RET, SMARCA4, BAP1*	0	1
Lung									2/2	*CCND2, CDK4, FGF23, MDM2, MLL2, PTEN, RB1, STK11, TP53*	0	0
Cervix									1/1	*AXIN1*	0	1

Table 4. Outcomes of genetic testing referrals.

ID	Prior Knowledge (Family/Personal)	Relevant history	Offered testing	Agreed to testing	Tumor NGS	Germline Testing	Comments
1	Yes, medical diagnosis of MEN1	Zollinger Ellison, Hyperparathyroidism, Insulinoma, Graves's	Yes	Yes	MEN1 R234fs*4	MEN1 c.682_685d up p.Arg229Hisfs*4	History was established
2	No	No	Yes	Yes	MEN1 Q96*	CHECK2 c.1427C>T p.Thr476Met	Heterozygous, VUS
3	No	No	Yes	Yes	MEN1 185fs*33	Negative	Also tested for HNPCC because of family history
4	Yes	Prolactinoma, parathyroid abnormalities, PanNEN	Yes	No	MEN K120del	Presumed MEN K120del	History was established
5	Yes	Hyperparathyroidism, bronchial carcinoid	Yes	No	MEN1 R234fs*4, NF1 loss	Presumed MEN1 R234fs*4	History was established
6	No	No	Yes	No	MEN1 185fs*33	N/A	Started but did not finish testing
7	Yes	Father and sister with MEN1, parathyroidectomy	Yes	No	MEN1 R532*	Presumed MEN1 R532*	Patient incarcerated
8	No	No	Yes	No	MEN1 Q171* TSC2 Q1588*	N/A	Second opinion
9	No	No	Yes	Yes	MEN1 R98fs*21	Negative	N/A
10	No	No	Yes	No	MEN1 Q171*	N/A	Financial concerns
11	No	No	Yes	Yes	MEN1 A572fs*29	Negative	N/A

12	No	No	Yes	No	MEN1 splice site 840-1G>C	N/A	N/A
13	No	No	Yes	Yes	MEN1 L22fs*94	Negative	N/A
14	No	No	Yes	No	MEN1 splice site 670-2A>C	N/A	Financial concerns
15	No	No	Yes	No	MEN1 Q541*, NF1 Y489C	N/A	Second opinion
16	No	No	Yes	Yes	MUTYH Y165C	Negative	N/A
17	No	No	Yes	No	MUTYH G382D	N/A	Pending (second opinion patient)
18	No	No	Yes	No	MUTYH splice site 1476+1_1476+2GT>AA	N/A	Patient changed their mind
19	No	No	Yes	No	TSC2 T1785fs*41, MEN1 Q7E	N/A	Pending
20	No	No	Yes	No	RET amplification §	N/A	Financial concerns
21	No	No	Yes	No	BRCA2 E2258*	N/A	Financial concerns

102 were deemed as potentially recruiting adult patients with neuroendocrine neoplasms. The eligible variants included *BRCA*, *MET* and *ATRX* with a list of most common mutations included in Table 2 (full data available on request). 52 of our 107 patients (48.5%) would not have been eligible for a clinical trial with reasons varying between no mutations in 26 patients (24%), sample failure in 9 patients (8.4%) or non-actionable mutations 17 patients (15.9%). Approximately 51.5% would in theory be eligible for an investigational treatment.

Discussion

In this study we explored the usefulness of NGS in an unselected cohort of metastatic patients with NENs treated in a tertiary institution. We exhibited that, while FDA approved tumor agnostic therapies were discovered in only 1-2% of our population, our patients demonstrated actionable mutations that could enlist them in clinical trials in approximately 52% of the cases. Moreover, around 23.1% of the patients would be eligible for germline testing based on tumor mutations such as *MEN1* and *BRCA* variants. This, it becomes clear that NGS has the potential to offer significant additional information including clinical trial eligibility and ideally should be obtained when evaluating a metastatic NEN patient.

Our observations are significant for the treatment of the rare NEN patient. Despite recent advances, treatment options for neuroendocrine tumors are still limited and are almost nonexistent for high grade tumors or pathologies such as adrenocortical cancers. Any additional treatment option can therefore offer the chance for a longer life, hopefully with better quality [19,20]. Previous publications have observed the mutational landscape of NENs, and their results are similar to ours, with some variations allowing for different tumor mixes. Park et al. performed NGS in 84 cases of gastrointestinal NENs and reported the most common mutations [21]. Their study identified *TP53, PIK3CA, RB1, KRAS, IDH-1*and *ATM*. Small bowel neuroendocrine tumors (Ki67 <2%) did not harbor any mutations. Vijayvergia et al. performed a similar study in poorly differentiated NENs and reported that the most frequent mutation was *TP53* (57%), followed by *KRAS* (30%), *PIK3CA/PTEN* (22%) and *BRAF* (13%) mutations [22]. Gleeson et al. performed NGS in pancreatic NENs and reported more than 20% of patients harboring more than two significant variants per tumor which the most prevalent being *MEN1 (42%), DAXX (11%), ATRX (10%),* and *TSC2 (8%)* [21,23].

Our study addressed the potential for addition of tumor agnostic treatments including immunotherapy and TRK fusion targeting agents. As previously described, we observed that, in general, low-grade tumors had fewer mutations compared with higher grade ones and that small bowel tumors tended to have fewer mutations and no *PD-L* expression. Only one patient had an *MSI*-H tumor but two had a high TMB, a surrogate for use of checkpoint inhibitors. Interestingly, our study identified that *PD-1* and *PD-L1* are highly expressed in both grade 1 and grade 3 but not in grade 2 NENs; this observation will be further addressed in a larger patient cohort. In our study we also found a *NTRK* mutation in one patient with unknown primary low grade NEN. While the patient has not been treated with anything more than somatostatin analogues because of indolent histology, a recent study demonstrated benefit of entrectinib (*NTRK* inhibitor) in a fusion positive neuroendocrine tumor which improved clinical symptoms while radiologic imaging showed pseudoprogression of tumor [24]. Our patient now has the added option of an NTRK inhibitor treatment and the opportunity to enroll in one of our institutional NTRK clinical trials, if and when they progress.

Moreover, this study also looked into the incidence of potentially actionable mutations and determined that it was higher in the higher grade or grade III NEC populations, something that has been reported before. While NEN specific clinical trials are sparse, we identified mainly Phase I/II trials around the world that, in an ideal setting, could have provided an option for an impressive 51.5% of our patients. This number is theoretical, as most NEN patients (including in our institution) do not get treated on a trial but is also an indication of the usefulness of NGS in providing valuable genetic information when it comes to screening. For the lucky or motivated NEN patient, this can be extremely important.

With regards to germline genetic testing, our study identified 24 potential germline mutations in 21 patients based on tumor NGS. All of the patients were offered genetic consultation. Five were confirmed either by sequencing of peripheral blood or very concordant clinical and family history. The data is unfortunately limited since almost half of the patients declined, did not follow up or had pending results at the time of this analysis. At the same time, a patient was screened for another hereditary nonpolyposis syndrome based on family history and another one had a germline *CHECK2* mutation of unknown significance. We continue to offer genetic testing to our patients and will publish updated results in a larger cohort.

Our study has significant limitations. This is an unselected group of metastatic patients who have presented at different stages of their treatment and have had various responses to classic agents. Most of these would not be eligible for a clinical trial due to disease stability, performance status, inability to travel and other. Moreover, even though we perused the extensive inclusion and exclusion criteria of clinicaltrials.gov, there is always the possibility that the studies identified would not be having slots available, accepting neuroendocrine patient subcategories or accepting the specific patients we had at the moment.

We view this attempt as more hypothesis generating and feel that it makes a point for the usefulness and applicability of NGS in a rare tumor population.

NENs are wonderfully complicated and do not fit one mold, therefore personalized therapies are bound to dominate the field. We expect that in the near future (5-10 years), the majority of NEN patients, both localized and metastatic, will by default undergo extensive profiling to determine the origin, familial component, aggressiveness and treatment options.

Conclusion and Relevance

Tumor NGS can point to familial syndromes in about 15% of patients and identify about 2% of patients who might benefit from tumor agnostic therapies. Moreover, about half of NEN patients have NGS abnormalities that can make them eligible for a clinical trial.

While clinical trials are not easily available for many patients and tumor NGS does not always prove a germline genetic mutation, this study argues that NGS in NENs can offer more options of treatment and insight in the disease process of a rare tumor.

References

1. Dasari, Arvind, Chan Shen, Daniel Halperin and Bo Zhao, et al. "Trends in the Incidence, Prevalence, and Survival Outcomes in Patients with Neuroendocrine Tumors in the United States." *JAMA Oncol* 3 (2017): 1335-1342.
2. Hallet, Julie, Calvin How Lim Law, Moises Cukier and Refik Saskin, et al. "Exploring the Rising Incidence of Neuroendocrine Tumors: A Population-Based Analysis of Epidemiology, Metastatic Presentation, and Outcomes." *Cancer* 121 (2015): 589-597.
3. Marx, Stephen, Allen M Spiegel, Monica C Skarulis and John L Doppman, et al. "Multiple Endocrine Neoplasia Type 1: Clinical and Genetic Topics." *Ann Int Med* 129 (1998): 484-494.
4. Donis-Keller, Helen, Shenshen Dou, David Chi and Katrin M Carlson, et al. "Mutations in the RET Proto-Oncogene are Associated with MEN 2A and FMTC." *Human Mol Genet* 2 (1993): 851-856.
5. Larson, AM, SS Hedgire, V Deshpande and AO Stemmer-Rachamimov, et al. "Pancreatic Neuroendocrine Tumors in Patients with Tuberous Sclerosis Complex." *Clin Genet* 82 (2012): 558-563.
6. Yao, James C, Manisha H Shah, Tetsuhide Ito and Catherine Lombard Bohas, et al. "Everolimus for Advanced Pancreatic Neuroendocrine Tumors." *New Eng J Med* 364 (2011): 514-523.
7. Raymond, Eric, Laetitia Dahan, Jean-Luc Raoul and Yung-Jue Bang, et al. "Sunitinib Malate for The Treatment of Pancreatic Neuroendocrine Tumors." *New Eng J Med* 364 (2011): 501-513.
8. Strosberg, Jonathan, Ghassan El-Haddad, Edward Wolin and Andrew Hendifar, et al. "Phase 3 Trial of 177Lu-Dotatate for Midgut Neuroendocrine Tumors." *New Eng J Med* 376 (2017): 125-135.
9. Solomon, Benjamin J, Tony Mok, Dong-Wan Kim and Yi-Long Wu, et al. "First-line Crizotinib Versus Chemotherapy in ALK-Positive Lung Cancer." *N Engl J Med* 371 (2014): 2167-2177.
10. Soria, Jean-Charles, Daniel SW Tan, Rita Chiari and Yi-Long Wu, et al. "First-Line Ceritinib versus Platinum-Based Chemotherapy in Advanced ALK-Rearranged Non-Small-Cell Lung Cancer (ASCEND-4): A Randomised, Open-Label, Phase 3 Study." *The Lancet* 389 (2017): 917-929.
11. Peters, Solange, D Ross Camidge, Alice T Shaw and Shirish Gadgeel, et al. "Alectinib versus Crizotinib In Untreated ALK-Positive Non–Small-Cell Lung Cancer." *New Eng J Med* 377 (2017): 829-838.
12. Cutsem, E Van, Heinz-Josef Lenz, CH Kohne and Volker Heinemann, et al. "Fluorouracil, Leucovorin, and Irinotecan plus Cetuximab Treatment and RAS Mutations in Colorectal Cancer." J Clin Oncol 33 (2015): 692-700.
13. Douillard, Jean-Yves, Kelly S Oliner, Salvatore Siena and Josep Tabernero, et al. "Panitumumab–FOLFOX4 Treatment and RAS Mutations in Colorectal Cancer." *New Eng J Med* 369 (2013): 1023-1034.
14. Drilon, Alexander, Theodore W Laetsch, Shivaani Kummar and Steven G DuBois, et al. "Efficacy of Larotrectinib in TRK Fusion–Positive Cancers in Adults And Children." *New Eng J Med* 378 (2018): 731-739.
15. Drilon, Alexander, Salvatore Siena, Sai-Hong Ignatius Ou and Manish Patel, et al. "Safety and Antitumor Activity of the Multitargeted pan-TRK, ROS1, and ALK Inhibitor Entrectinib: Combined Results from Two Phase I Trials (ALKA-372-001 and STARTRK-1)." *Cancer Disc* 7 (2017): 400-409.
16. Marcus, Leigh, Steven J Lemery, Patricia Keegan and Richard Pazdur. "FDA Approval Summary: Pembrolizumab for the Treatment of Microsatellite Instability-High Solid Tumors." *Clin Cancer Res* 25 (2019): 3753-3758.
17. Lemery, Steven, Patricia Keegan and Richard Pazdur. "First FDA Approval Agnostic of Cancer Site-When a Biomarker Defines the Indication." *New Eng J Med* 377 (2017): 1409-1412.
18. Backman, Samuel, Olov Norlen, Barbro Eriksson and Britt Skogseid, et al. "Detection of Somatic Mutations in Gastroenteropancreatic Neuroendocrine Tumors using Targeted Deep Sequencing." *Anticancer Res* 37 (2017): 705-712.
19. Garcia-Carbonero, Rocio, H Sorbye, E Baudin and E Raymond, et al. "ENETS Consensus Guidelines for High-Grade Gastroenteropancreatic Neuroendocrine Tumors and Neuroendocrine Carcinomas." *Neuroendocrinol* 103 (2016): 186-194.
20. Oronsky, Bryan, Patrick C Ma, Daniel Morgensztern and Corey A Carter. "Nothing but NET: A Review of Neuroendocrine Tumors and Carcinomas." *Neoplasia* 19 (2017): 991-1002.
21. Park, Ha Young, Mi Jung Kwon, Ho Suk Kang and Yun Joong Kim, et al. "Targeted Next-Generation Sequencing of Well-Differentiated Rectal, Gastric and Appendiceal Neuroendocrine Tumors to Identify Potential Targets." *Human Pathol* 87 (2019): 83-94.
22. Vijayvergia, Namrata, Patrick M Boland, Elizabeth Handorf and Karen S Gustafson, et al. "Molecular Profiling of Neuroendocrine Malignancies to Identify Prognostic and Therapeutic Markers: A Fox Chase Cancer Center Pilot Study." *British J Cancer* 115 (2016): 564-570.
23. Gleeson, Ferga C, Jesse S Voss, Benjamin R Kipp and Sarah E Kerr, et al. "Assessment of Pancreatic Neuroendocrine Tumor Cytologic Genotype Diversity to guide personalized medicine using a Custom Gastroenteropancreatic Next-Generation Sequencing Panel." *Oncotarget* 8 (2017): 93464.
24. Sigal, Darren, Marie Tartar, Marin Xavier and Fei Bao, et al. "Activity of Entrectinib in a Patient with the First Reported NTRK Fusion in Neuroendocrine Cancer." *J National Compreh Cancer Network* 15 (2017): 1317-1322.

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