Practical experience and outcomes of implementation of multiplatform molecular profiling in routine clinical practice for patients with rare or refractory cancer at Parisian hospitals

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Abstract

Background: Early outcome data describing the use of comprehensive multiplatform tumor profiling (MP) in routine clinical practice has been encouraging. MP can provide treatment options which may not have been otherwise considered for patients with rare cancers or in whom standard of care options have been tested and failed. The feasibility and application of this multiplatform approach in France has not yet been tested.

Materials and Methods: This retrospective multi-center, observational evaluation was conducted on patients with refractory or progressing metastatic solid tumors, whose tumor biopsy samples were sent for MP between August 2011 and February 2015. Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis. Metrics of testing performance and patient treatment selection and outcomes were collected.

Results: Biopsy material from 74 patients was sent for MP using Caris Molecular Intelligence® (CMI). A median 10 treatments in 5 drug classes were associated with potential benefit and 14 treatments were associated with potential lack of benefit. 80% of treatments associated with potential benefit were cytotoxic chemotherapies that were readily accessible. Sequencing was attempted in 58 patients. At least one mutation was found in 39 patients, with 8 being wild-type. The most common mutations found were in the TP53, KRAS, APC and PIK3CA genes. Exceptional outcomes were reported in a number of patients using treatments that would not otherwise be considered, including a patient with refractory metastatic ovarian cancer who was treated successfully with trastuzumab.

Conclusions: The results of this evaluation show that broad tumour profiling can be implemented in routine clinical practice and is a tool, which can aid physicians in selection of treatment in difficult cases. The majority of treatments used were cytotoxic chemotherapies. Further prospective evaluation of the approach is warranted.

Background

- A pilot study has shown that comprehensive molecular profiling can be used to find molecular targets in patients with refractory metastatic cancer. In 18 of 66 patients treated with a molecularly guided therapy, the approach resulted in a longer PFS on an MP-suggested regimen than on the prior regimen on which the patient had just experienced progression. Exploratory analysis demonstrated that this PFS ratio correlated with the clinical parameter of overall survival.[1]
- A recent study in patients with refractory breast cancer showed that tumor profiling resulted in a revision of the original treatment decision for all patients and tumor profiling-based therapy resulted in a clinical benefit in 52% of heavily pretreated patients.[2]
- Similar outcomes were recently reported in pancreatobiliary cancer (clinical benefit in 37.5%) and adenoid cystic carcinoma (response in 4/11) patients treated in line with tumor profiling results in Israel[3, 4]
- A review of all patients treated in a single center in Australia resulted in clinical and survival benefits in over half of the patients and confirmed the role of molecular profiling in a clinical practice setting.[5]
- The aim of this study was to retrospectively assess the impact of using molecular profiling to guide treatment choice in patients with rare or refractory cancer in routine clinical practice at key hospitals in the Paris area.

Methods

- 74 patients with rare or refractory cancer being treated at Hôpital américain de Paris, CHU Treichville or Groupe hospitalier universitaire Pitié-Salpêtrière - Charles Foix were referred to Caris Life Sciences for comprehensive tumor profiling between August 2011 and February 2015.
- Specific testing was performed on tumor biopsy samples from all patients per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis.
- IHC analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies.
- Fluorescent in-situ hybridization (FISH) was used for evaluation of the HER-2/neu [HER-2/CEP17 probe], EGFR [EGFR/CEP7 probe], and cMET [cMET/CEP7 probe] (Abbott Molecular/Vysis). HER-2/neu and cMET status were evaluated by chromogenic in-situ hybridization (INFORM HER-2 Dual ISH DNA Probe Cocktail; commercially available cMET and chromosome 7 DIG probe; Ventana). The same scoring system was applied as for FISH.
- Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform. Specific regions of 45 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.

Demographics

- 74 patients in total (35 female, 39 male), 6 with insufficient material
- Average age = 59.6 yo (median 60 yo, range 24-81)
- Average time to testing from biopsy = 328.6 days (median 104 days, range 14-1306)

Primary Site	n	Primary Site	n	Primary Site	n
Lung	14	Ovary	4	Connective tissue	2
Pancreas	9	Prostate gland	4	Gastroesophageal junction	2
Breast	8	Gastric	3	Liver	2
Unknown primary site	5	Kidney	3	Ampulla of Vater, Anus, Appendix, Biliary Tract, Brain, Cervix uteri, Eye, Intrahepatic bile duct, Testis, Thymus, Tonsil, Uterus	1
Colon	4	Skin	3		

Feasibility of CMI Testing

- Average CMI Turnaround time in Lab = 10.6 calendar days (median 9 days; range 5 – 29 days)
- The report provided actionable information in all cases. 96% of patients (65/68) had at least one treatment associated with potential benefit and 99% (67/68) had at least one treatment associated with potential lack of benefit.
- A median of 10 treatments across 5 drug classes were associated with potential benefit while a median of 14 drugs in 5 drug classes were associated with potential lack of benefit.

Tests and Treatments Associated with Potential Benefit

	IHC	ISH	NGS	Total Treatments	Drug Classes	Cytotoxic (n, %)	Hormone (n, %)	Targeted (n, %)
Average	6.8	0.5	0.4	11.2	4.8	8.4 (80.14%)	2.1 (11.63%)	0.8 (8.23%)
Median	7	0	0	10	5	9	0	0
Min-Max	0-11	0-2	0-4	0-25	0-10	0-15	0-16	0-5

Tests and Treatments Associated with Potential Lack of Benefit

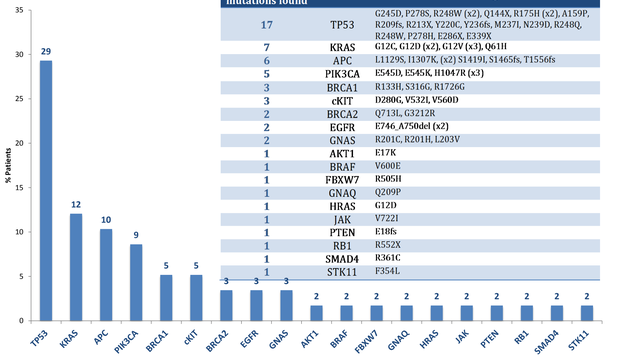
	IHC	ISH	NGS	Total Treatments	Drug Classes	Cytotoxic (n, %)	Hormone (n, %)	Targeted (n, %)
Average	4.6	1.3	1.3	14.6	4.8	4.2 (34.84%)	6.0 (28.21%)	4.5 (36.95%)
Median	4	1	1	14	5	4	0	5
Min-Max	0-12	0-5	0-5	0-29	0-11	0-12	0-18	0-12

	Potential Benefit	Potential Lack of Benefit	Indeterminate benefit
Antimetabolites *(5-FU, pemetrexed, capecitabine)*	49	13	0
Nucleoside analog *(gemcitabine)*	48	10	0
Taxanes *(docetaxel, paclitaxel)*	48	10	0
Topoisomerase inhibitors *(irinotecan, topotecan)*	41	18	1
Albumin-bound taxane *(Nab-paclitaxel)*	33	6	20
Anthracyclines *(doxorubicin, epirubicin, liposomal-doxorubicin)*	31	31	0
Alkylating agents *(temozolomide, dacrabazine)*	25	35	0
mTOR inhibitors *(everolimus, temsirolimus)*	11	5	32
Hormone therapy	10	28	0
EGFR monoclonal antibodies *(cetuximab, panitumumab)*	8	6	1
Androgen deprivation therapy	5	26	0
HER2-directed therapy *(trastuzumab, T-DM1, pertuzumab, lapatinib)*	5	47	11
Platinum agents *(cisplatin, carboplatin, oxaliplatin)*	3	4	27
EGFR TKIs *(erlotinib, gefitinib)*	3	6	3
2nd generation EGFR TKIs *(afatinib)*	2	5	1
BRAF inhibitors *(vemurafenib, dabrafenib)*	1	33	5
Small molecular kinase inhibitor *(imatinib)*	0	3	45
ALK inhibitor *(crizotinib)*	0	12	0

Cytotoxic Chemotherapy **Hormone Therapy** **Targeted Therapy**

Results of Next Generation Sequencing Analysis

- Sequencing was attempted in 58 patients, 11 tests were QNS
- Tumors with at least one mutation = 39, wildtype tumors = 8
- Average number of genes with alterations = 1.2 (median 1, range 0-3)
- Average number of genes tested with no alterations found = 39.9 (median = 43, range 0 – 46)
- Average number of genes tested with indeterminate results = 3.3 (median 1, range 0-44)



Number of mutations found	Gene	Protein Changes Found
17	TP53	G245D, P278S, R248W (x2), Q144X, R175H (x2), A159P, R209fs, R213X, Y220C, Y236fs, M237I, N239D, R248Q, R248W, P278H, E286X, E339X
7	KRAS	G12C, G12D (x2), G12V (x3), Q61H
6	APC	L1129S, I1307K, (x2) S1419I, S1465fs, T1556fs
5	PIK3CA	E545D, E545K, H1047R (x3)
3	BRCA1	R133H, S316G, R1726G
3	cKIT	D280G, V532I, V560D
2	BRCA2	Q713L, G3212R
2	EGFR	E746_A750del (x2)
2	GNAS	R201C, R201H, L203V
1	AKT1	E17K
1	BRAF	V600E
1	FBXW7	R505H
1	GNAQ	Q209P
1	HRAS	G12D
1	JAK	V722I
1	PTEN	E188fs
1	RB1	R552X
1	SMAD4	R361C
1	STK11	F354L

Study Highlights – Responses to Off-Label Trastuzumab Use

- Three patients treated with off-label trastuzumab based on HER2 protein overexpression had excellent responses

NSCLC Cancer Patient Treated with trastuzumab based on HER2 IHC and ISH Result

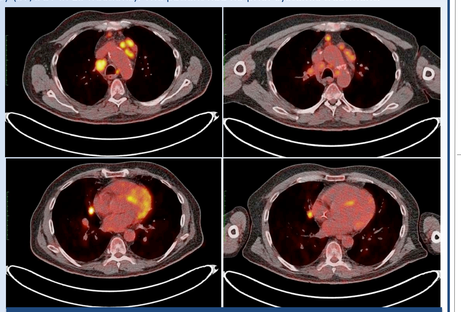
67 year old male with metastases of adenocarcinoma pointing to a bronchial origin and mediastinal adenopathy. Patient had a medical history of non-insulin dependent diabetes, renal insufficiency, deep vein thrombosis, appendectomy, hypertension and smoking (35 pack years).

Treatment History

The patient had prior radiotherapy (37/5GY in 15 fractions). The patient had no prior systemic treatment.

CMI performed

- Based on the equivocal HER IHC result (2+ in 60% of cells and gene amplification by ISH (2.04) as well as TLE3 positivity, the patient began treatment with trastuzumab and paclitaxel.
- After two months, the overall tumor volume was reduced with a partial response observed.
- Progression of disease in the thorax occurred after 5 months.



Ovarian Cancer Patient Treated with trastuzumab based on HER2 IHC Result

48 year old female (history of peritonitis, hepatitis A and smoking (less than 10 pack-years)) was diagnosed with a poorly differentiated high grade serous carcinoma of the left ovary in Oct 2010. Family history of breast cancer and ovarian cancer.

Treatment History

Oct 2010 - Neoadjuvant chemotherapy (carboplatin, paclitaxel) cycles.
Dec 2010 - Omentectomy, pelvic and para-aortic lymphadenoectomy.
Feb 2011 – Recurrent disease. Treated with gemcitabine, oxaliplatin, bevacizumab (6 cycles).
Apr 2012 – Recurrence in mediastinal lymph node treated with surgery and carboplatin, paclitaxel. Complete response in FDG-PET and CA-125.
Aug 2013 – Elevated CA 125 (65 U/mL) with new hilar lymphadenopathy.
Feb 2014 – Elevation CA 125 (116 U/mL) with bilateral hilar and adrenal left with peritoneal carcinomatosis. Treated with gemcitabine, oxaliplatin, bevacizumab.
Sep 2014 - Elevation CA-125 (145U/mL) treated with liposomal doxorubicin.
CMI performed
Jan 2015 – Increase in CA125 to 1400 U/mL and volume of left hilar lymphadenopathy, adrenal and pararectal nodules.

Treatment begun with trastuzumab and paclitaxel based on HER2 IHC (3+ staining in 80% of cells) and ISH (ratio of 6.36). After two months, the overall tumor volume in the thorax had reduced by 80-90% in the abdomen by 95%.

Ampullary Cancer Patient Treated with Trastuzumab based on HER2 IHC Results

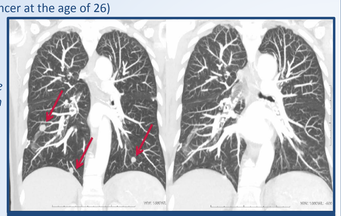
59 year old female diagnosed with ampullary lesion in May 2011. Familial history of cancer (brother: pancreatic adenocarcinoma at the age of 64; daughter: ovarian cancer at the age of 26)

Treatment History

May 2011 – Duodenopancreatectomy
Nov 2011 – Multiple (>10) lung nodules observed
- FOLFOX (3 Months) - *initial response, poor tolerance*
- FOLFOX (1 month), LV5FU2 (2 months) - *progression*

July 2012 – Progression of lung nodules
- FOLFIRI (6 months) – *stable disease*
- Gemcitabine – *initial response*

April 2013 – CMI performed
- Trastuzumab and paclitaxel selected based on HER2 IHC (3+ staining in 90% of cells) and TUBB3 loss
- After 3 months of treatment, objective partial response with tumor regression of > 80% observed.



Conclusions

- Implementation of broad tumor profiling is feasible in France with results provided in a timely manner which fit with routine clinical practice.
- The majority of treatments associated with benefit are cytotoxic agents.
- Sequencing alone provided at least one mutation in 67% of cases, but the most commonly observed mutations were not good targets for today's commercially available or investigational drugs.
- Tumor profiling can identify treatments that would not otherwise have been considered and can lead to exceptional response. This evaluation illustrated the potential for off-label trastuzumab in carefully selected patients.

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