Prospective next generation sequencing (NGS) of rare or poor prognosis cancers

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RUTGERS Cancer Institute of New Jersey

NCI CCC A Comprehensive Cancer Center Designated by the National Cancer Institute

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Abstract

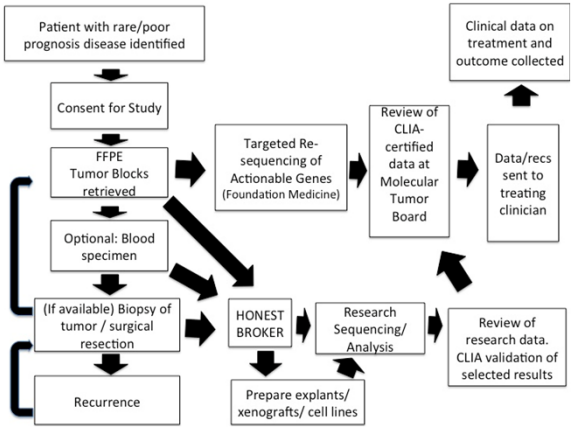
Background. Genomic screening for somatic alterations in individual tumors is informative for understanding tumor biology and identifying novel treatment approaches and research opportunities. We initiated a research study where patients with rare or poor prognosis cancers were enrolled and underwent targeted sequencing using a CLIA certified assay. Sequencing results were reviewed at a molecular tumor board and therapeutic strategies were suggested if appropriate. Preliminary results of this study are now presented.

Experimental procedures. We successfully analyzed the genomic profiles of formalin-fixed paraffin embedded tumor specimens from 92 patients using the FoundationOne™ (Foundation Medicine, Cambridge, MA) platform for targeted sequencing of the entire coding sequence of 236 genes and 47 introns of 19 genes involved in fusions in a CLIA-certified CAP accredited laboratory. A range of evaluated tumor subtypes of epithelial and mesenchymal origin were evaluated. The majority of cases were from rare or uncommon histologic classes, though recurrent/refractory cases of colon cancer and triple negative breast cancers were also enrolled. Both clinical history and sequencing data were presented at a multidisciplinary molecular tumor board for development of therapeutic recommendations.

Results. At least one genomic alteration was found in 85 cases (92%) and none in 7 cases (8%). Serial specimens were obtained in 8 patients. Seven had gain of at least one mutation in serial specimens. The average number of mutations identified was 3.6 (range 0-10). The most common genomic alterations detected were in p53 (45%), KRAS (17%), PI3K (20%) and PTEN (14%). Alterations in the FGF pathway were surprisingly common (8% receptor and 12% FGF alterations). A number of mutations occurred at low frequency but included an additional large set of potentially actionable genes: ALK, ERBB2, BRCA2, MET. The majority of cases had mutations for which our molecular tumor board had recommended action: either potential enrollment in clinical trial or off label use of approved therapy. NGS sequencing results led to implemented clinical action in ~20% of cases. Another 20% remain actionable in the future.

Schema

Fig 1. Prospective NGS trial schema



Genomic Profiles

Table 1. Demographics of 100 patients and tumor characteristics.

Gender	
Male	37
Female	63
Race	
Caucasian	76
African American	13
Hispanic	8
Asian	3
Mean age, years	56.5
Age range, years	3.7-81.3
Stage at study entry (AJCC TNM)	
M1	91
M0	9
Tumor Type	
Breast (epithelial, phyllodes)	14
Upper GI (GE/esophageal, gastric, pancreas)	16
Hepatobiliary	5
Colorectal	9
GU (RCC, urothelial, prostate, bladder)	9
Gynecologic (ovary/fallop tube, uterine, cervix)	15
Sarcoma/GIST	10
Thyroid	5
Neuroendocrine	5
Thoracic (NSCLC, mesothelioma)	3
Skin (melanoma, squamous, basalosquamous)	4
CUP (epithelial)	2
Other	3

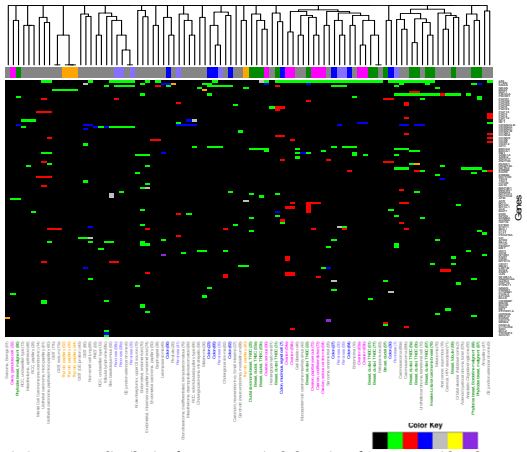


Fig 2. Heat map distribution from unsupervised clustering of 85 tumors with at least one genomic alteration. Color-coded top bar and lower labels define tumor types. Alterations: black=none, green=mutation, red=amp, blue=del, grey=splice, yellow=fusion, purple=rearrangement.

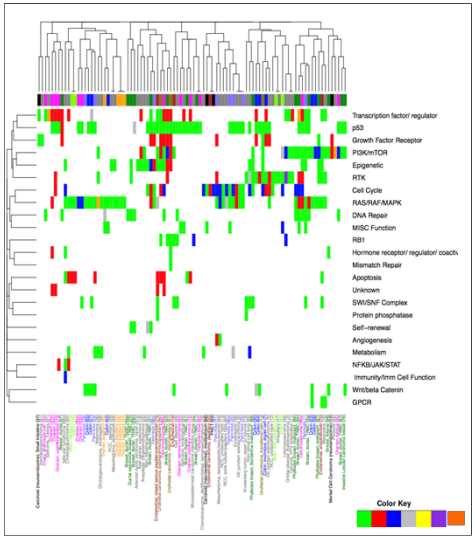


Fig 3. Bidirectional clustering of 85 tumors by alterations in gene pathways. Alterations: white=none, green=mutation, red=amp, blue=del, grey=splice, yellow=fusion, purple=rearrangement, orange= multiple different alterations.

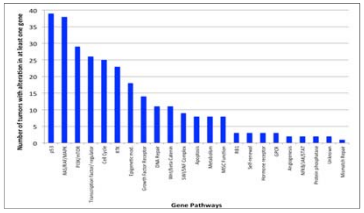


Fig 4. Mutational landscape from 85 independent tumors defined by gene pathways.

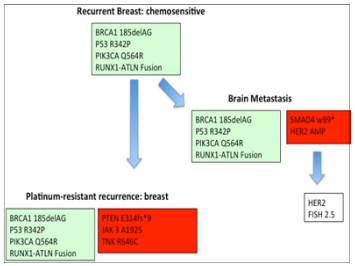


Fig 5. Evolution of a BRCA1-mutant, triple negative breast cancer.

Clinical Action

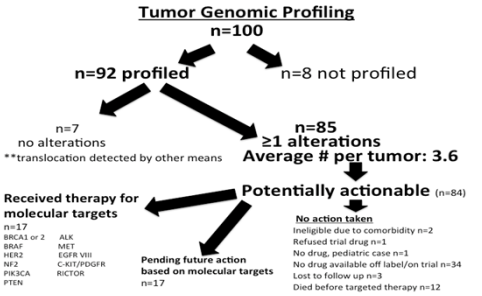


Fig 6. Tumor genomic profiling resulted in successful targeted sequencing with at least one alteration in the majority of cases. A significant number of alterations resulted in molecularly-based therapeutic recommendations.

Tumor type	Alteration	Clinical action
Gallbladder cancer	BRCA2	• Germline testing • Continued therapy platinum salt • Recommendation for enrollment in PARP inhibitor trial
Colon cancer	ALK possible activating mutation	• Patient enrolled on early phase trial of ALK inhibitor • Mutation being validated in laboratory
Iodine-refractory papillary thyroid cancer	BRAF V600E	Treatment with vemurafenib: documented response, clinical improvement (lasting several months)
Renal cell cancer	Activating mutation MET	MET inhibitor crizotinib recommended/enrolled in clinical trial
Urothelial cell cancer	Activating mutation HER2	Trial of HER2 inhibitor (lapatinib) with transient clinical response
Cervical cancer (HPV+)	PIK3CA mutation	05L105-Dose Escalation dose Level 0, MK-2206 and HCQ
TNBC	BRCA2 mutation	AC ABTB88 started 7/20/12
Renal cell cancer	NF2 splice site 1574+2T>A	Initiated temsirolimus
Ovarian cancer	BRCA1 mutation, BRCA2 truncation	NCI/CTEP 8282: A Phase 1 Study of Chronically-Dosed, Single-Agent ABT-888 in Patients with Either BRCA 1/2 Mutated Cancer; Platinum-Refractory Ovarian, Fallopian Tube, or Primary Peritoneal Cancer; or Basal-Like Breast Cancer
Krukenberg tumor	EGFR VIII	Initiated afatinib
Carcinosarcoma (initial dx: GIST and SCC)	Lacked C-KIT or PDGFR mutation	• Discontinued imatinib • Patient died suddenly before starting platinum-based therapy
Merkel Cell Carcinoma	RICTOR amplification	mTOR inhibitor
GIST, recurrent	Lacked C-KIT or PDGFR mutation	Discontinued imatinib
Colon cancer	PIK3CA E545A	TED dual PI3K and mTOR inhibitor
Endometrial cancer	PIK3CA H1047R; PTEN Y336*	Initiate everolimus
Ovarian cancer	BRCA1 Q1240*	veliparib M13-695 ph 1
GIST, recurrent	KIT V559A	regorafinib

Table 2. Representative tumor types, actionable genomic alterations, and therapeutic action.

Conclusions

- Prospective comprehensive clinical NGS assay identifies actionable genomic alterations with relevant targeted therapies.
- Serial specimen analysis provide clues to tumor evolution and resistance mechanisms.
- An institutional molecular tumor board is an effective venue to systematically review sequencing data and generate clinical recommendations.
- Knowledge of genomic alterations results in referral to clinical trials, FDA-approved therapy, off label use of targeted therapy, and planning of trials for agents targeting identified genes/pathways.
- Challenges to therapeutic implementation include:
  - lack of practical access to clinical trials
  - limited availability of targeted agents
- Expanded trial portfolios, NGS earlier in clinical course, and increased access to targeted agents will increase actionability.

Acknowledgements

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