SUPPLEMENTARY FILE to manuscript:

Standardization of EUS-guided FNB technique for molecular profiling in pancreatic cancer: Results of a randomized trial

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IRB/Registration

The study was approved by the institutional review board of the hospital (IRB No. 1741103) and registered at ClinicalTrials.gov (NCT05043532).

Specimen processing

After specimen procurement and formation of formalin-fixed paraffin-embedded cell blocks, the purified DNA and RNA were eluted separately to quantify nucleic acid. NGS was performed (Archer Panels) for analyzing 69 gene DNA mutations (single nucleotide variants, insertions, and deletions) and 53 RNA somatic oncogenic gene fusions.

Randomization and Masking

Computer-generated randomization assignments were provided by the statistician using a block randomization method (block randomization of 4) and placed in sequentially numbered, sealed, opaque envelopes. Patients with pancreatic mass proven to have adenocarcinoma by rapid onsite evaluation at EUS were randomized equally (1:1 allocation) to two or three dedicated passes for comprehensive molecular profiling.

Sample size calculation and Statistical analysis

A two-sided sample size calculation was performed based on the proportion of specimens with adequate tissue to enable molecular profiling. Assuming presence of adequate tissue to allow molecular profiling in 50% of patients in the two-pass group and in 90% in the three-pass group[1-3], the sample size was estimated at 16 per group (total sample size of 33 to account for a 5% drop out rate), at 80% power and two-sided alpha of 0.05.

Continuous data were summarized as means with standard deviation or medians with interquartile range and range, and were compared using the Student's t-test or Wilcoxon rank-sum test as indicated.

Categorical data were summarized as frequencies with percentages and were compared using the chi-square or the Fisher's exact test as indicated.

Additional Comments

Germline testing is important in pancreatic cancer because it is associated with hereditary syndromes involving multiple generations. For such families, screening is recommended. However, screening does not detect all persons at risk. The Memorial Sloan Kettering IMPACT study that conducted germline testing of more than 1,000 patients with cancer revealed a high incidence (17%) of mutations in the pancreatic cancer subset, of whom 42% had no family history of cancer and would not have met current screening recommendations.[4] Although the main driver mutation is KRAS, there are numerous other potentially actionable mutations that can be identified using molecular profiling.[5] Also, from a diagnostic standpoint, there is growing evidence that when cytology and histology are inconclusive, molecular testing can aid diagnosis.[3]

In a recently published study in which 25G needles were used and 2 passes were performed for tissue procurement, only 84% of FFPE specimen yielded adequate DNA.[6] In two previous randomized trials, we have shown that fanning the needle and performing stylet-retraction when using the Franseen needle yielded best cellularity.

References

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Supplemental Table 1. Details of the mutations identified in 33 pancreatic ductal adenocarcinoma patients from comprehensive molecular profiling

Randomization-FNB passes	RNA Concentration	DNA Concentration	Variants Detected 1	Mutations 1	Variants Detected 2	Mutations 2	Variants Detected 3	Mutations 3	Notes
three	25.7	7.56	KRAS	p.Gly12Val	RB1	p.Arg320Ter	TP53	p.Thr155Pro	TERT G373A
two	3.57	0.874	KRAS	p.Gly12Asp					
two	3.98	2.79	KRAS	p.Gly12Asp	KRAS	p.Gln61His			
three	10.2	2.47	KRAS	p.Gln61Leu					
three	35.2	8.07	KRAS	p.Gly12Val					
two	10.4	2.76	KRAS	p.Gly12Asp					TERT G373A
three	13.7	16	KRAS	p.Gly12Asp	PIK3CA	p.Glu545Ala	TP53	p.Arg175His	
two	TOO LOW	8.35	none detected	TERT G49C					
two	72	20	KRAS	p.Gly12Asp	TP53	p.Arg175His			TERT G349C
three	24	6.99	KRAS	p.Gly12Asp					
three	9.9	8.94	KRAS	p.Gly12Asp	TP53	p.Tyr163Cys			
two	47.3	13	KRAS	p.Gly12Asp	TP53	p.Arg196Ter			
three	38.3	13.8	KRAS	p.Gly12Arg					
two	40.1	7.21	KRAS	p.Gly12Asp	GNAS	p.Arg201His			
two	32.8	11.9	KRAS	p.Gly12Val					
three	46.8	6.23	KRAS	p.Gly12Val	TP53	p.Tyr205His			
two	5.8	2.6	KRAS	p.Gly12Val	TP53	p.Cys242Phe			
two	12.3	2.4	KRAS	p.Gly12Cys					
three	49	14	KRAS	p.Gly12Asp	TP53	p.Arg175His			
three	39.8	2.56	KRAS	p.Gly12Asp	TP53	p.Arg273Cys			
three	40.1	9.69	KRAS	p.Gly12Val	TP53	p.Arg175His			
two	53	20.3	KRAS	p.Gly12Val					
three	36.5	11.9	KRAS	p.Gly12Val	TP53	p.Arg282Trp			
two	32.5	8.29	KRAS	p.Gly12Val	TP53	p.Cys176Ser			
two	52	20.9	TERT	T349C					
three	TOO LOW	0.68	KRAS	p.Gly12Val	SMAD4	p.Arg445Ter			
two	57	16.6	KRAS	p.Gly12Asp	TERT	T349C			
two	27.2	9.64	KRAS	p.Gln61His	TP53	p.Tyr163Asn			
three	15.2	4.2	KRAS	p.Gly12Val					
three	17.9	8.07	KRAS	p.Gly12Arg	TP53	p.Cys176Arg			
three	30.5	5.69	none detected	NONE DETECTED					RNA FUSION POSITIVE
two	47.2	14.3	TP53	p.Pro278Arg	TERT	T349C	BRCA1	p.Gln356Ter	
two	97	20.5	KRAS	p.Gly12Val					

Supplemental Figure 1. CONSORT flow diagram of patient enrollment