Molecular Characterization of Advanced Muscle Invasive Bladder Cancer (MIBC): Comparison of Low-Input Targeted RNA Sequencing (RNAseq) Strategies.

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BACKGROUND

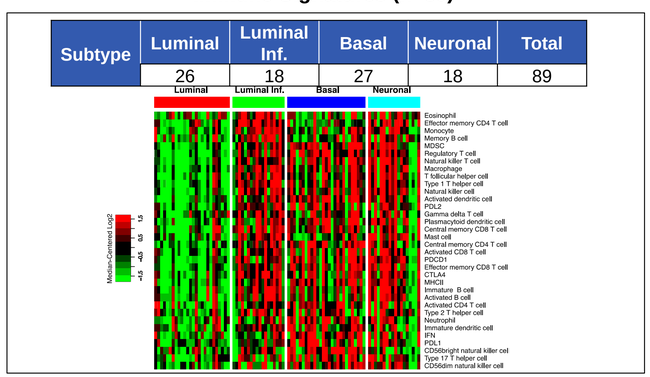
- 5-year survival for advanced MIBC is poor (~5%), but newer targeted agents (e.g., PD-1/PD-L1 or FGFR) have improved survival over traditional chemotherapy.
- To better select patients suited for targeted therapies, molecular characterization is more important than ever, but the amount of tumor material available can limit options.
- The following study compares RNAseq using a low-RNA-input (SureSelect hybridization-capture: 200 ng extracted RNA) and an ultra low-RNA-input (AmpliSeq PCR amplification: 10 ng extracted RNA) method as part of the "BACI" retrospective study of 109 advanced MIBC patients treated with anti-PD-1/PD-L1 therapy (Mayhew et al, 2019 ASCO).
- RNAseq results, including correlation between methods and molecular subtype agreement are presented herein.

METHODS

- RNA extracts from 24 representative FFPE MIBC tumor samples from the 109 patients were analyzed using SureSelect XT RNA (Agilent) and AmpliSeq transcriptome (Illumina) by NovaSeq6000 paired end sequencing.
- Previously, 89 out of the 109 "BACI" samples were used to call subtypes using the GeneCentric 60-gene MIBC nearest centroid subtyper following SureSelect XT RNA sequencing, which has been shown to identify potential immune differences by molecular subtype (Figure 1; Mayhew et al, 2019 ASCO).
- AmpliSeq test samples were evenly split across 4 molecular subtypes (luminal, luminal infiltrated, basal, and neuronal) based on the subtyping conducted on SureSelect RNAseq.
- Pearson correlation analysis was performed for all genes, as well as the 60 subtyper genes.
- Expression subtype calls were compared using RNAseq data obtained from both sequencing methods.

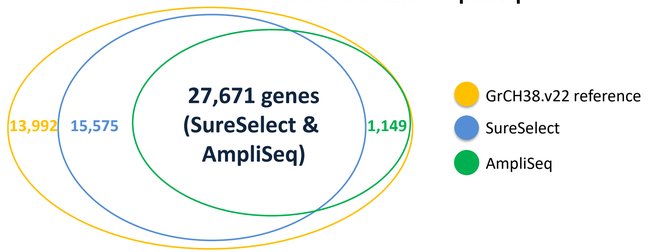
Figure 1. 60-Gene MIBC Nearest Centroid Subtyper Using SureSelect: Immune Signatures (n=89)

Subtype	Luminal	Luminal Inf.	Basal	Neuronal	Total
	26	18	27	18	89



RESULTS

Figure 2. Overlap of genes mapping to GrCH38 Human Reference between SureSelect and AmpliSeq



- 58,000 genes were queried (GrCH38.v22) for the comparison between the SureSelect and AmpliSeq methods for the n=24 samples (Figure 2):
  - Average number of PE reads (millions): SureSelect 90.7, AmpliSeq 57.5
  - 27,671 (55%) were found using both methods
  - 13,992 (28%) were not found using either method
  - 15,575 unique genes were found using SureSelect
  - 1,149 unique genes were found using AmpliSeq
- The two methods were well correlated: median of 0.87 (0.73-0.89) overall and 0.80 (0.54-0.86) for the 60 subtyper genes (Figure 3).

Figure 3. Correlation Analysis Between SureSelect and AmpliSeq

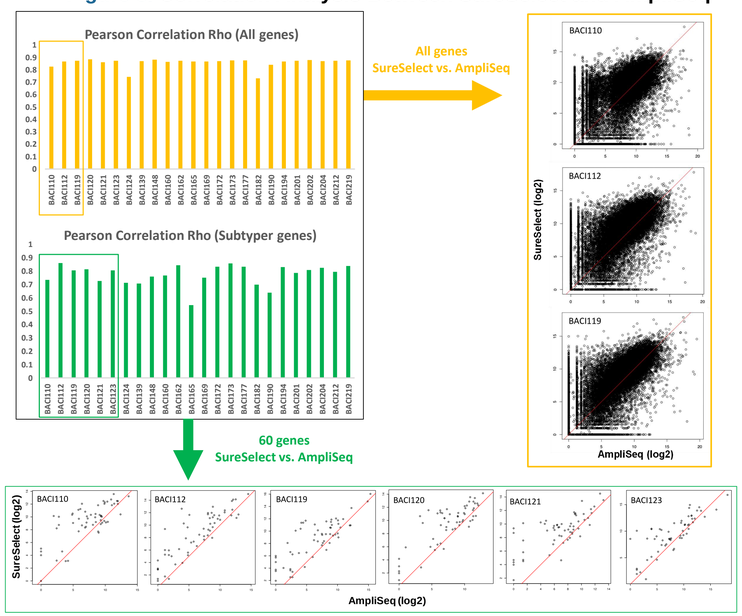
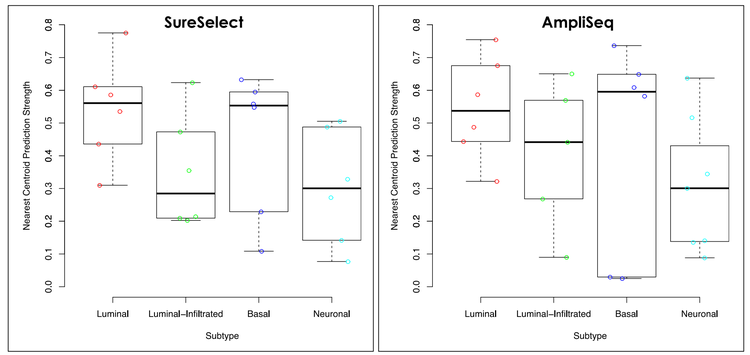


Table 1. Subtype Call Comparison: SureSelect vs. AmpliSeq

Subtype	Luminal	Luminal Inf.	Basal	Neuronal	Total
SureSelect	6	6	6	6	24
AmpliSeq	6	5	6	7	24

- 22 of 24 subtype calls were congruent between SureSelect and AmpliSeq (Table 1)
  - One Luminal Infiltrated call in SureSelect was called Basal in AmpliSeq
  - One Basal call in SureSelect was called Neuronal in AmpliSeq
  - Cohen's kappa agreement = 0.889 (p-value = 4.04e-14)

Figure 4. MIBC Subtype Call and Prediction Strength Comparison: SureSelect vs. AmpliSeq



- Nearest centroid subtype prediction strengths for SureSelect and AmpliSeq (Figure 4)
  - Calculated by the difference between the distance to the nearest and second-nearest subtype centroids
  - High subtype prediction strength correlation between both methods at 0.82

SUMMARY AND CONCLUSIONS

- This study demonstrated comparable RNAseq analysis using both a low-input and an ultra-low FFPE-compatible method.
- Using AmpliSeq, MIBC tumors were able to be molecularly characterized, including subtype analysis, with only 1/20th of the extracted RNA needed for SureSelect.
- There is potential to use RNAseq data for MIBC subtyper calling from either method, allowing for analysis of samples that were previously rejected for low (<200 ng) RNA content.
- Further validation of AmpliSeq methodology in MIBC and other tumor types is warranted.

REFERENCES

1. Mayhew et al, J Clin Oncol 2019; 37, no. 15 suppl (May 20, 2019) 4558-4558

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