



Identification and Characterization of an Intra-Tumoral Microbiome in Soft Tissue Sarcomas

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Background

- ✓ Immunotherapy is the “fourth pillar” of cancer treatment (Nobel Prize in 2018 by Drs. Allison and Honjo).
- ✓ The gut microbiome has been shown to impact immunotherapy outcomes in cancer.¹
- ✓ In human-to-mouse experiments involving fecal transplantation (FMT), the effect of the gut microbiome on immunotherapy outcomes was observed to be transferable.¹
- ✓ Cancer patients who receive antibiotics prior to initiation of immunotherapy may have worse outcomes, which is hypothesized to be due to the negative impact of antibiotics on the gut microbiome.²
- ✓ Microbiota have also been identified in solid tumors of the pancreas and breast—organs that communicate with the outside world.^{3,4}
- ✓ In a murine model of pancreatic cancer, the intra-tumoral microbiome was shown to promote inhibitory immune pathways, and antibiotics reversed this effect.⁴

1. V. Gopalakrishnan et al. *Science* 2018;359:97-103
 2. D. Pinato et al. *JAMA Oncol.* 2019;5(12):1774-1778
 3. C. Urbaniak et al. *Appl. Environ. Microbiol.* 2016;82(16):5039-5048
 4. S. Pushalkar et al. *Cancer Discov.* 2018;8:403-416

Research Questions

- 1) Is there a soft tissue sarcoma (STS) microbiome?
- 2) If so, is it clinically relevant?
 - Impact on immune phenotype (stimulatory vs. inhibitory)?
 - Correlation with the gut microbiome?
 - Response to cancer therapy?
 - Prognosis?

Methods

- 16S rRNA sequencing was employed to identify microbiome-specific genetic signatures in eight treated (post-radiotherapy) archived STS specimens (**Table 1**), obtained from the UC Davis Comprehensive Cancer Center Biorepository.
- Abundance and diversity of microbial organisms were examined against a background of reagent-only negative controls.

Results

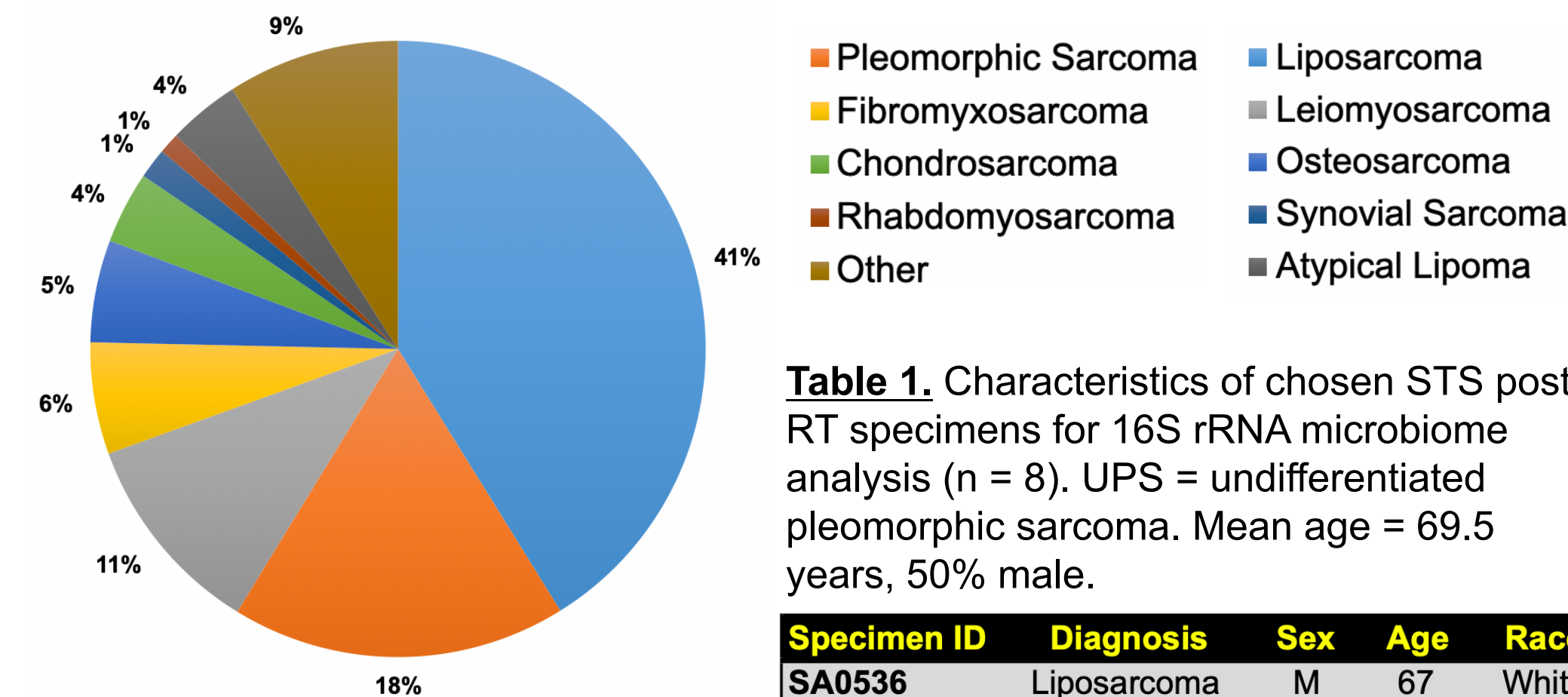


Figure 1. Overview of banked patient sarcoma tissue at the UC Davis Comprehensive Cancer Center Biorepository, classified by sarcoma sub-type (n = 186).

Table 1. Characteristics of chosen STS post-RT specimens for 16S rRNA microbiome analysis (n = 8). UPS = undifferentiated pleomorphic sarcoma. Mean age = 69.5 years, 50% male.

Specimen ID	Diagnosis	Sex	Age	Race
SA0536	Liposarcoma	M	67	White
SA0553	UPS	M	65	White
SA0648	UPS	F	49	White
SA0737	UPS	F	86	White
SA0738	UPS	F	86	White
SA0970	UPS	M	86	White
SA1210	Liposarcoma	F	54	White
SA1216	UPS	M	63	White

Table 2. Relative abundances of the six most abundant families of bacteria in each patient STS sample, expressed as percentages of the total 16S reads per sample.

FAMILY	SA0536 (% of 33,614)	SA0553 (% of 46,143)	SA0648 (% of 42,483)	SA0737 (% of 42,292)	SA0738 (% of 71,789)	SA0970 (% of 51,659)	SA1210 (% of 43,179)	SA1216 (% of 57,148)
<i>Bacillaceae</i>	65.5%	35.8%	1.0%	63.3%	58.3%	47.2%	61.6%	54.7%
<i>Enterobacteriaceae</i>	0.0%	38.0%	0.4%	6.9%	0.0%	11.4%	0.0%	0.0%
<i>Enterococcaceae</i>	0.0%	3.5%	37.3%	0.2%	0.0%	1.1%	0.0%	0.0%
<i>Tannerellaceae</i>	0.0%	3.0%	23.7%	2.1%	0.0%	4.3%	0.0%	0.0%
<i>Lachnospiraceae</i>	0.0%	5.0%	19.0%	1.2%	0.0%	7.5%	0.0%	0.0%
<i>Burkholderiaceae</i>	29.2%	1.9%	0.1%	7.8%	27.3%	5.4%	28.9%	33.6%
<i>Staphylococcaceae</i>	2.4%	2.9%	1.2%	3.0%	9.9%	1.8%	5.1%	3.4%
Other	2.9%	9.8%	17.3%	15.6%	4.5%	21.4%	4.4%	8.4%
TOTAL	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 3. Relative abundances of the seven most abundant genera of bacteria in each patient STS sample, expressed as percentages of the total 16S reads per sample.

GENUS	SA0536 (% of 33,614)	SA0553 (% of 46,143)	SA0648 (% of 42,483)	SA0737 (% of 42,292)	SA0738 (% of 71,789)	SA0970 (% of 51,659)	SA1210 (% of 43,179)	SA1216 (% of 57,148)
<i>Bacillus</i>	31.5%	21.4%	0.5%	31.9%	27.5%	27.3%	33.3%	25.4%
<i>Anaerobacillus</i>	34.1%	14.4%	0.5%	31.4%	30.9%	19.8%	28.3%	29.3%
<i>Escherichia/Shigella</i>	0.0%	38.0%	0.4%	6.9%	0.0%	11.4%	0.0%	0.0%
<i>Enterococcus</i>	0.0%	3.5%	37.3%	0.2%	0.0%	1.1%	0.0%	0.0%
<i>Parabacteroides</i>	0.0%	3.0%	23.7%	2.1%	0.0%	4.3%	0.0%	0.0%
<i>Blautia</i>	0.0%	1.6%	12.3%	0.8%	0.0%	2.5%	0.0%	0.0%
<i>Ralstonia</i>	29.2%	1.9%	0.1%	7.8%	27.3%	5.4%	28.9%	35.6%
<i>Staphylococcus</i>	2.4%	2.8%	1.2%	3.0%	9.9%	1.8%	5.1%	3.4%
Other	2.8%	13.3%	24.1%	15.9%	4.5%	26.3%	4.4%	6.4%
TOTAL	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

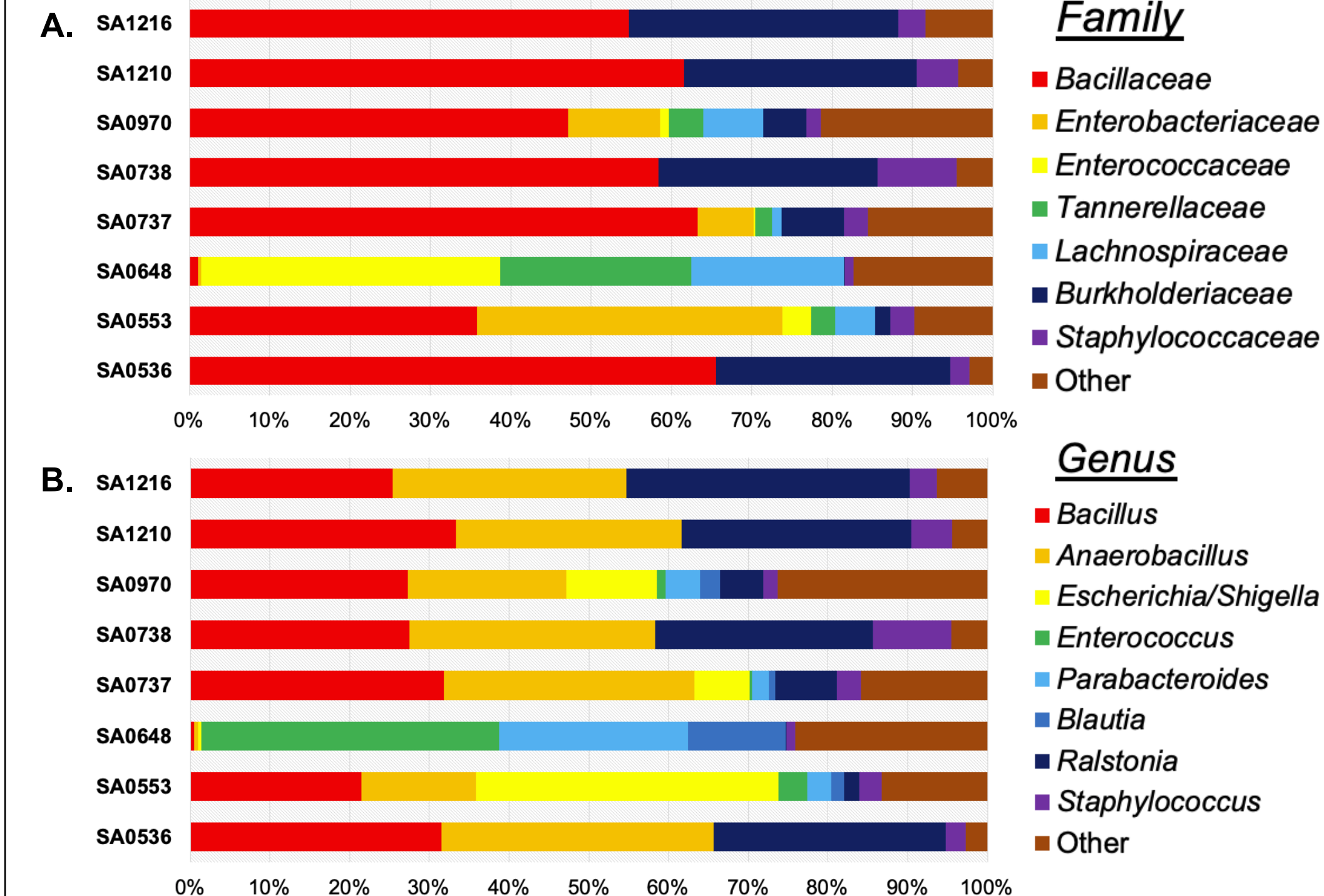


Figure 2 (A-B). Stacked bar plots at the family (A) and genus (B) level showing the relative abundance of each family and genus per patient STS sample, respectively.

Conclusion & Future Directions

- We report exciting preliminary data demonstrating, for the first time to our knowledge, the existence of an intra-tumoral microbiome in STS.
- Validation is ongoing, including a prospective collection and assessment of tumor tissue alongside key clinical correlates (**Figure 4**).
- If our hypotheses are correct, it would be an exciting opportunity for innovation in the treatment of this cancer that poses a formidable challenge in disseminated disease.

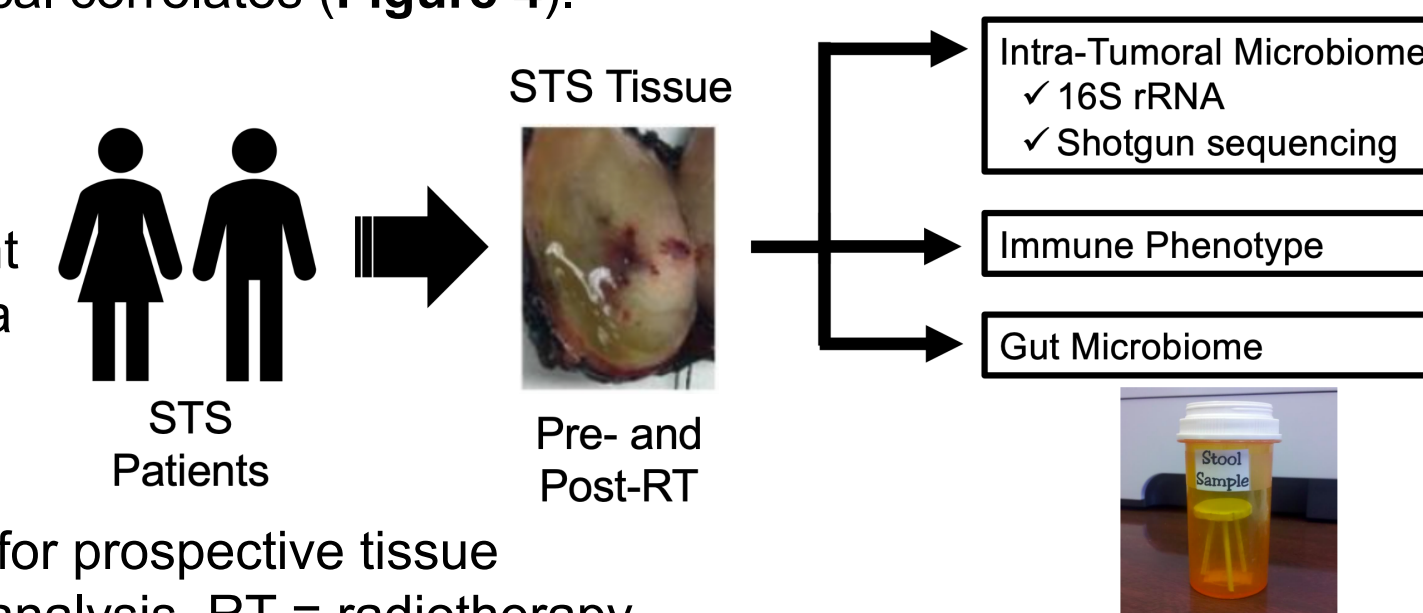


Figure 3. Study schematic for prospective tissue collection and microbiome analysis. RT = radiotherapy.

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