Integration of Clinical and Molecular Biomarkers for Skin Melanoma Survival: Challenges and opportunities identified through the Biospecimen Core at MSK

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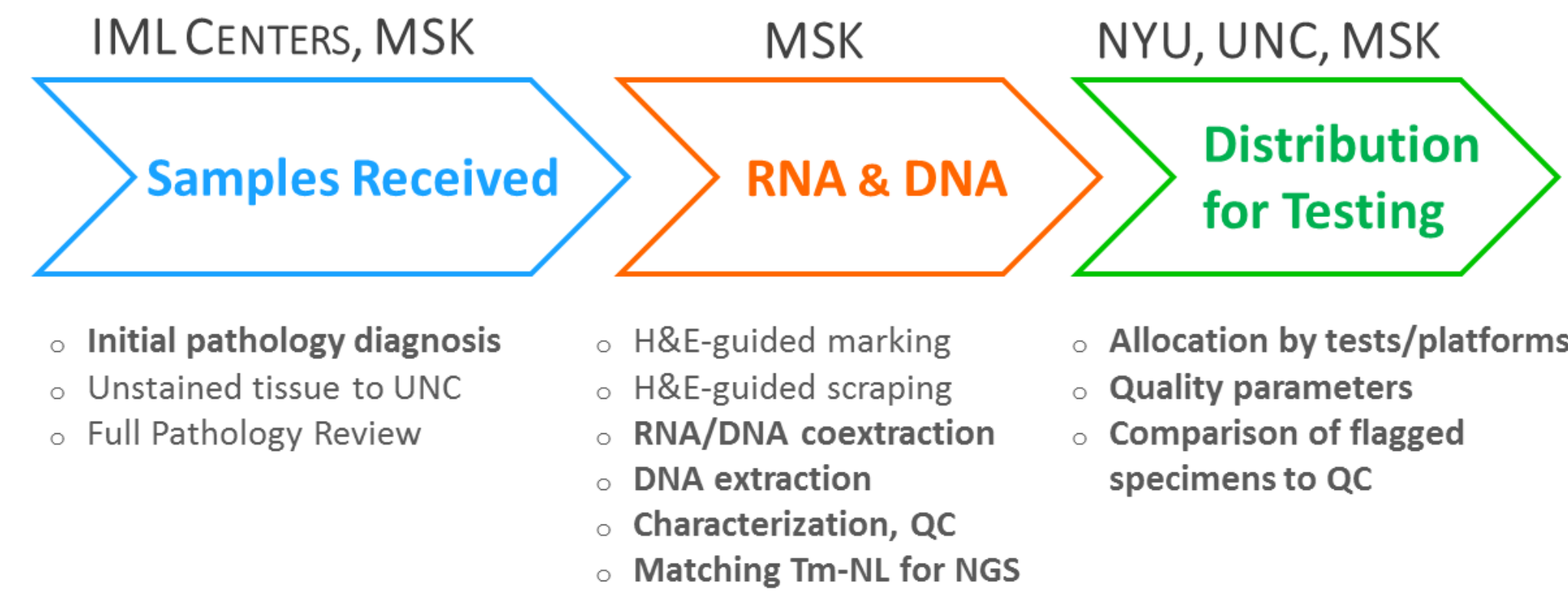**Memorial Sloan Kettering Cancer Center**

Background

While most melanomas are localized at presentation, heterogeneity exists in patients' outcomes and treatment-responses. We are currently conducting a multicenter study on primary melanomas to identify robust classifiers predictive of melanoma-specific survival using multiple omics-platforms (P01CA206980).

The Aims of the MSK Biospecimens Core (BC) for this P01 are to establish the optimal collection and handling of archived paired primary melanomas/normal tissue, confirm diagnosis, perform histology-guided co-extraction of nucleic acids (NA), distribute well-characterized quality-NA for genetic and epigenetic testing, and immune-profiling in 1000 patients identified through the international InterMEL consortium. Here, we present the progress and unique aspects of optimizing a multicenter study of small-size pigmented tumors.
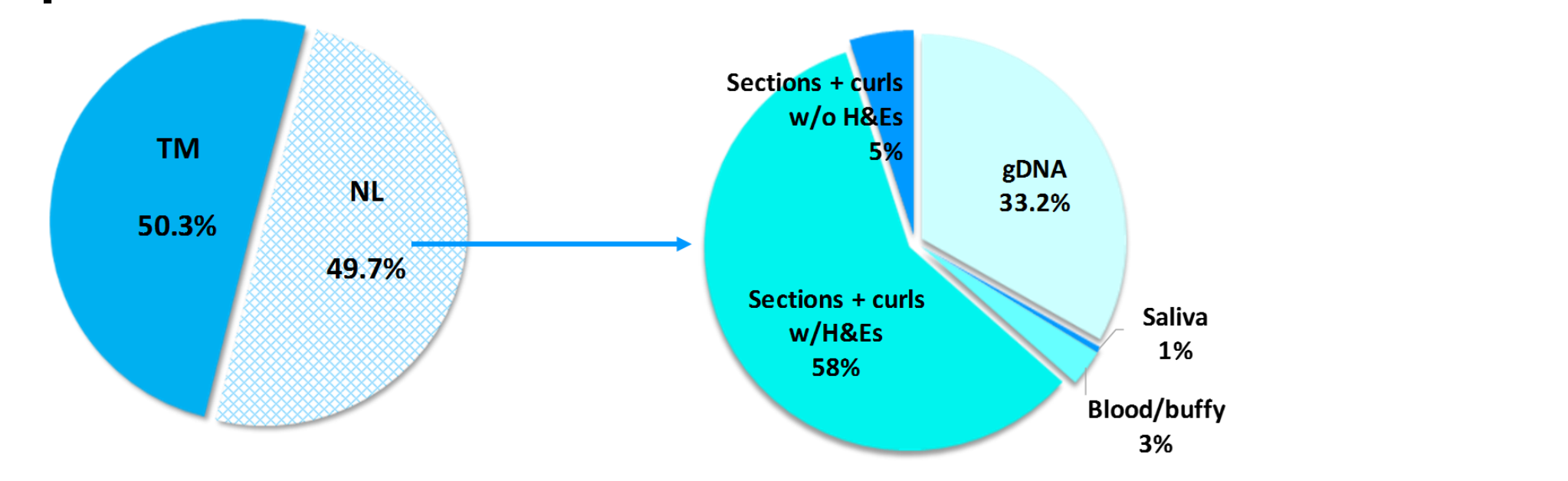
Methods

- Tumor and Normal tissues from melanoma patients stages II/III (50% survived within 5 years)
- 9 centers contribute with 2xH&Es (for diagnosis+), 7x10μm FFPE sections (for DNA/RNA extractions), unstained FFPE sections (for IHC)
- RNA & DNA samples are co-extracted from common lysates (Qiagen ALLprep)
- Tumor-RNAs are distributed for miRNA-characterization, tumor-DNAs for methylation-profiling, and paired tumor-normal DNA for mutation screening with MSK-IMPACT™
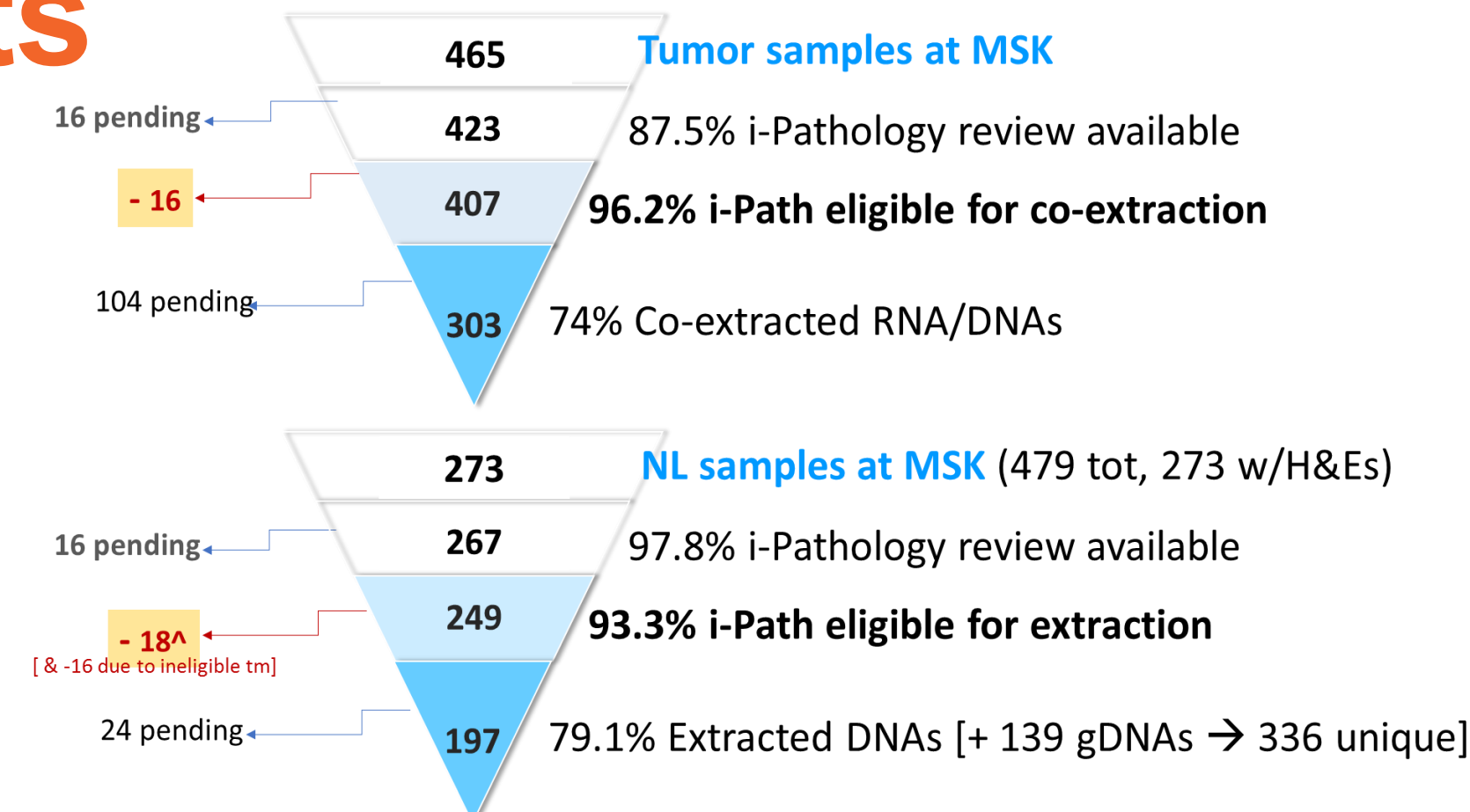
Overview of the workflow

IML CENTERS, MSK → Samples Received
- Initial pathology diagnosis
- Unstained tissue to UNC
- Full Pathology Review

MSK → RNA & DNA
- H&E-guided marking
- H&E-guided scraping
- RNA/DNA coextraction
- DNA extraction
- Characterization, QC
- Matching Tm-NL for NGS

NYU, UNC, MSK → Distribution for Testing
- Allocation by tests/platforms
- Quality parameters
- Comparison of flagged specimens to QC

Samples received to date

TM 50.3% / NL 49.7%

Sections + curls w/o H&Es 5%; gDNA 33.2%; Sections + curls w/H&Es 58%; Saliva 1%; Blood/buffy 3%

Results

- **465** Tumor samples at MSK
- **423** 87.5% i-Pathology review available (16 pending)
- **407** 96.2% i-Path eligible for co-extraction (- 16)
- **303** 74% Co-extracted RNA/DNAs (104 pending)

- **273** NL samples at MSK (479 tot, 273 w/H&Es)
- **267** 97.8% i-Pathology review available (16 pending)
- **249** 93.3% i-Path eligible for extraction (- 18^ [&-16 due to ineligible tm])
- **197** 79.1% Extracted DNAs [+ 139 gDNAs → 336 unique] (24 pending)

Total Tumor RNA obtained per patient

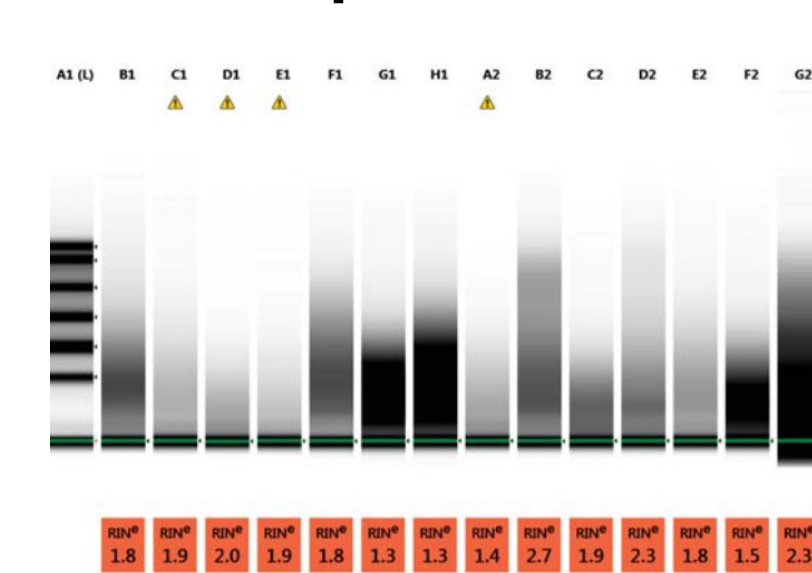|	Nanodrop^			Tape Station*			
	Tot ng	A260/280	A260/230	Tot ng	RIN	Fraction >200nt (%)	Tot ng >200nt
Mean	11047	1.9	0.8	2552	2	45.1	1162
Median	**6134**	**1.9**	**0.8**	1535	**2**	**45.6**	**558**
Range	344 – 101,905	1.3-2.5	0-1.9	216 – 4032	1-4	7.9 -100	7.2-10,255

Values are for sum of Elution 1 & 2; *TapeStation values are for Elution 1 only; nt, nucleotides; n-10 RNAs failed, were not remeasured to avoid degradation
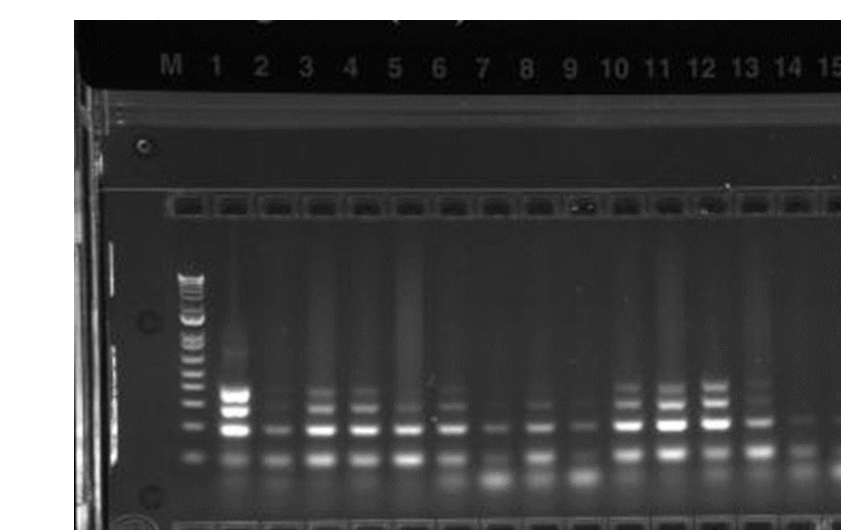
Total Tumor DNA obtained per patient

	Nanodrop (tot DNA)^			Qubit ~ (dsDNA)	% dsDNA	QC-PCR
	Tot ng	A260/280	A260/230	Tot ng		# bands
Mean	5,933	1.9	1.0	1599	27	2.2
Median	**3,570**	**1.8**	**0.8**	**821**	27	**2.5**
Range	86 – 64,852	1.3-8.8	0-13.2	17-25,842	2.9-58	0-4

^ values are for sum of Elution 1 & 2. ~ Qubit values mostly Elution 1.% dsDNA = fraction of dsDNA (100 x dsDNA/totDNA). QC-PCR values are for Elution 1 and/or 2; bands correspond to amplicon sizes 100 to 400bp. Smears were recorded.
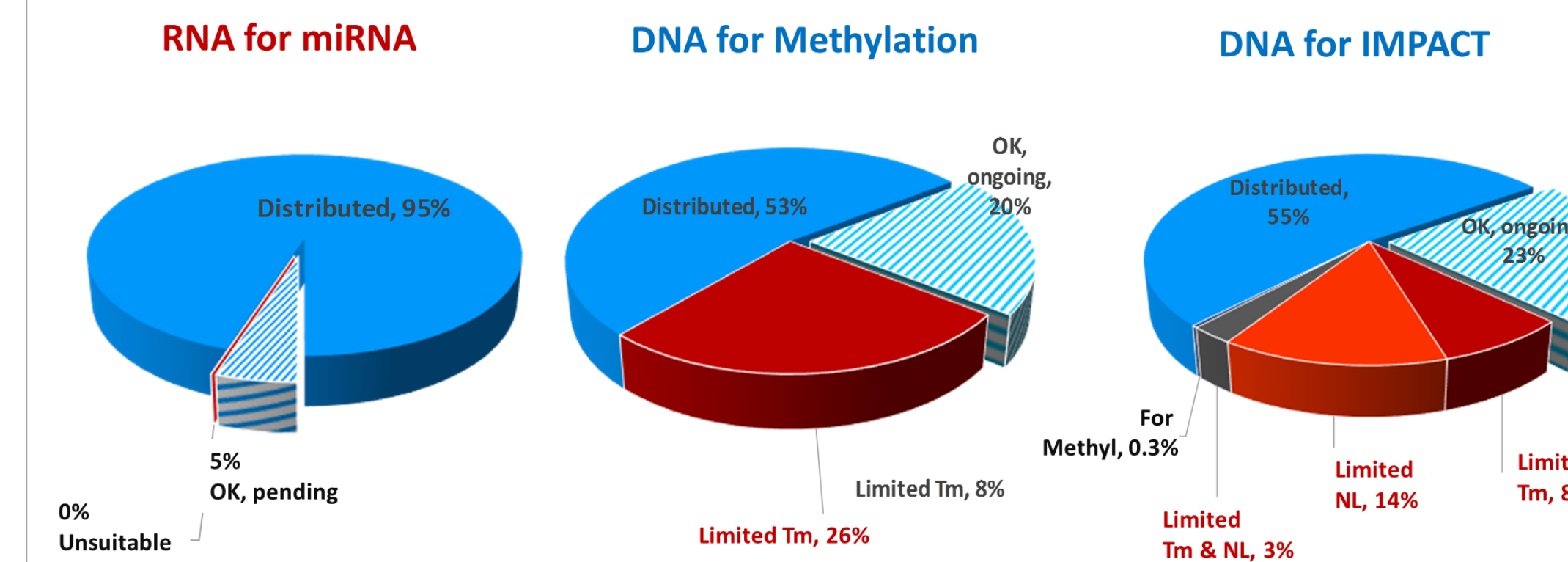
Quality assessment of RNA: TapeStation

Quality assessment of DNA: QC-PCR

QC-PCR targets amplicons of 100bp, 200bp, 300bp, and 400bp

Distribution of samples for testing by different platforms

RNA for miRNA: Distributed, 95%; 5% OK, pending; 0% Unsuitable

DNA for Methylation: Distributed, 53%; OK, ongoing, 20%; Limited Tm, 8%; Limited Tm, 26%

DNA for IMPACT: Distributed, 55%; OK, ongoing, 33%; For Methyl, 0.3%; Limited NL, 14%; Limited Tm & NL, 3%; Limited Tm, 8%

- 288 (~95.1%) Tm RNA for miRNA expression
- 161 (~53.1%) Tm DNA for methylation
- 156 (~51%) Tm DNA for MSK-IMPACT™

RNA and DNA samples distributed for testing by all 3 platforms for 121 InterMEL cases

Discussion:

In this ongoing study, we continue to:

Receive specimens, extract and co-extract NL-DNA and Tm RNA/DNA.

Adjust conditions (e.g., elution volumes) and include additional quality parameters as more data form the different genetic and epigenetic platforms become available.

Distribute additional RNAs for miRNA, tm DNA for Methylation, and NL-Tm paired DNAs for MSK-IMPACT™ testing

In addition:

We will provide additional feedback to centers to guide them with the procurement/sectioning of adequate tissues; cellularity and quality of tissue (no scaring, no necrosis) are stressed

NL-DNA will be extracted from the surrounding of Tm tissues as needed and when initial pathology review deems it appropriate.

We will evaluate QC parameters specific to each testing platform against our sample-specific features