Proposed translational correlates of ReLaPSe (Prospective study evaluating the treatment outcomes for localized recurrent, resectable retroperitoneal liposarcoma)

On behalf of the Basic and Translational Science Working Group

(Too) Many Unanswered Questions in LPS

1. What are the differences and similarities between WD and DD components of a mixed primary/recurrent WD/DD tumor?

2. What is the extent of intratumoral heterogeneity between different regions within a primary/recurrent WD and/or DD component of LPS?

3. How does LPS evolve from primary presentation to first recurrence? And how is tumor evolution affected by systemic therapy

4. Do WD in a WD-only LPS differ from WD in a mixed primary/recurrent WD/DD LPS?

5. What changes are induced by preoperative RT, in the tumor and in the peripheral immune system? And timing of changes?

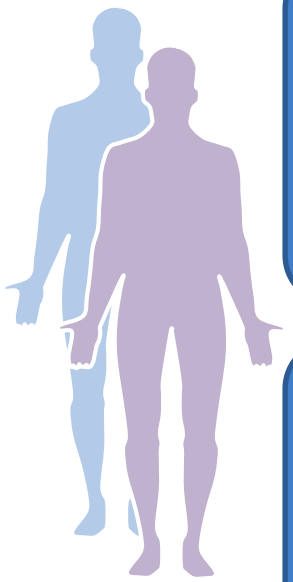
6. What changes are induced by preoperative systemic therapy, in the tumor and in the peripheral immune system? And timing of changes?

Prospective study evaluating the treatment outcomes for localised recurrent, resectable retroperitoneal liposarcoma (ReLaPSe)

Study population:
Localized, recurrent, resectable retroperitonel and/or pelvic LPS

Key inclusion:
- Age ≥ 18
- Prior R0/R1 resection
- ASA < 3
- WHO PS ≤ 2
- Adequate organ function
- Recurrence deemed likely resectable (R0/R1)
- All disease deemed treatable by RT

Key exclusion:
- Prior RT
- Contraindication for RT



Cohort 1: Surgery with preoperative RT (any chemo) N ≥ 40

Cohort 2: Surgery, no preop RT (any chemo) N ≥ 40

Any chemo, +/- preop RT → Surgery → Follow-up q3-6 months for 5 years

Pre-treatment biopsy
Imaging

Imaging (within 4 weeks of surgery)

Tumor tissue

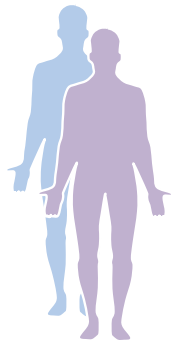
Design: prospective, non-randomized
Sample size: 100 patients (at least 40 in each cohort)
Enrollment duration: 36 months
Primary Objective: Abdominopelvic recurrence-free survival (ARFS)
Secondary Endpoint: Overall survival , cumulative incidence of 2nd local recurrence, cumulative incidence of distant metastasis, pathologic response (EORTC-STBSB criteria), radiolographic response (RECIST 1.1, Choi), cumulative incidence of in-field relapse (preop RT group only), local and distant disease progression during preop RT, toxicity of preop RT, QoL, unplanned R2 resection, use of chemotherapy, validation of recurrent RPS nomogram

What questions could be examined within ReLaPSe design?

1. What are the differences and similarities between WD and DD components of a mixed ~~primary/~~recurrent WD/DD tumor?

2. What is the extent of intratumoral heterogeneity between different regions within a ~~primary/~~recurrent WD and/or DD component of LPS?

3. How does LPS evolve from primary presentation to first recurrence? And how is tumor evolution affected by systemic therapy (if corresponding primary tumor were previously banked)



Untreated patients proceeding to surgery alone

Cohort 1: S…rv…th pre…ve …em… N ≥ 40

Cohort 2: Surgery, no preop RT (any chemo) N ≥ 40

Any chem… …preop RT → Surgery → Follow-up q3-6 months for 5 years

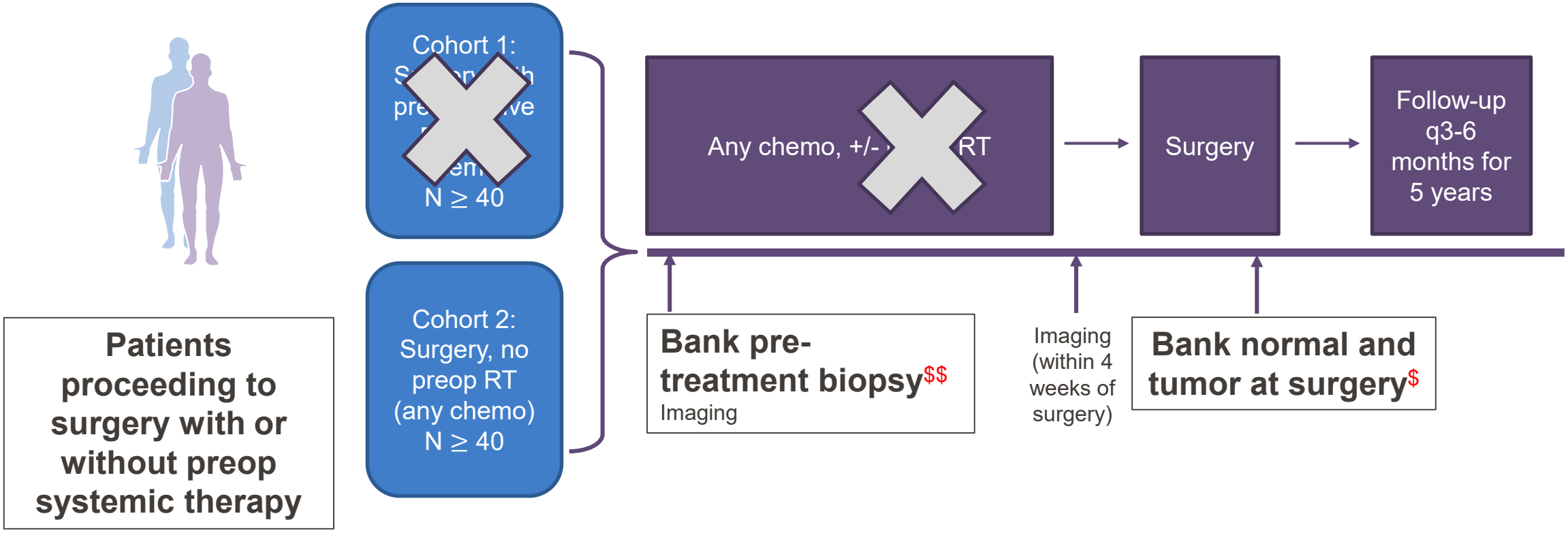
Pre-treatment biopsy Imaging

Imaging (within 4 weeks of surgery)

Bank normal and tumor at surgery$

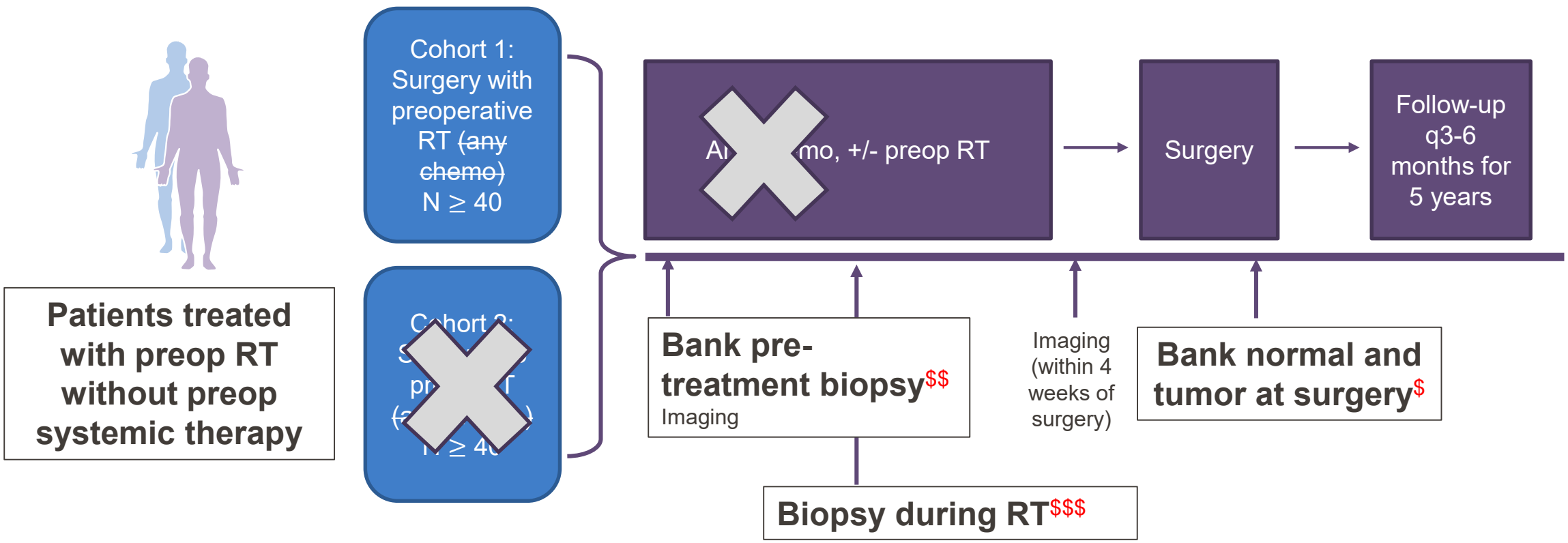
What questions could be examined within ReLaPSe design?

4. Do WD in a WD-only LPS differ from WD in a mixed ~~primary/~~recurrent WD/DD LPS?



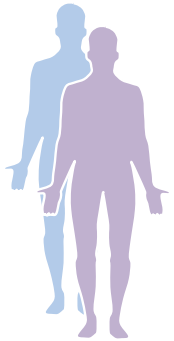
What questions could be examined within ReLaPSe design?

5. What changes are induced by preoperative RT, in the tumor and in the peripheral immune system? And timing of changes?



What questions could be examined within ReLaPSe design?

6. What changes are induced by preoperative systemic therapy, in the tumor and in the peripheral immune system?



Patients treated with preop systemic therapy without preop RT

Cohort 1: [illegible] pre[illegible]ive [illegible]emo
N ≥ 40

Cohort 2: Surgery, no preop RT (any chemo)
N ≥ 40

Any chemo, +/- [illegible] RT → Surgery → Follow-up q3-6 months for 5 years

Bank pre-treatment biopsy$$
Imaging

Imaging (within 4 weeks of surgery)

Bank normal and tumor at surgery$

Proposed biospecimens to bank

Prioritisation (4–6 cores)	Distribution
FFPE	1/3
Snap frozen	1/3
RPMI or fresh → single cell suspensions	1/3*

Tube type	Volume drawn	Derivative
Sodium heparin or EDTA	20 mL	PBMCs, plasma
Procoagulant with or without gel separator	10 mL	Serum
Streck or sodium citrate	10mL	Double spun plasma for cfDNA analysis†

Logistics/Practicalities

- Will need SOP for all sites for specimen acquisition and banking

- Sample storage and processing: will biospecimens be
  - Stored and processed locally then generated data be aggregated and analysed
  - Stored locally then shipped to central locations for processing and data generation
  - Collected locally then shipped to central locations for storage

- Funding…

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THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Proposed biospecimens to bank

1. Tumor features we could interrogate – and this can each be done at the bulk tumor, single-cell, or spatial level, we'd have to prioritize.

a. Genome complexity (chromosomal rearrangements, acquisition of mutations)

- **(1, 2) HiC – frozen**
- **(1, 2) WGS – frozen better, FFPE possible**
- **(1, 2) WES (can be done using same library as WGS)**

b. Chromatin accessibility

- **(1, 2) H3K27Ac [I haven't had luck with ATAC-seq for LPS, not sure if others have. H3K27Ac ChIP-seq can be a surrogate]**
- **(1, 2) DNA methylome [I think there are technologies that could do this at same time as long read DNA sequencing, Nanopore for instance]**

c. Transcriptome

- **(1, 2, 3) RNA – frozen best, FFPE for spatial**

d. Immune infiltrates

- **(1, 2) RNA signature**
- **(3) IHC, mIF, spatial CyToF, spatial transcriptomics**

Unanswered Questions in LPS

1. What is the extent of intratumoral heterogeneity between different regions within a WD and/or DD component of LPS?
(A) Compare sample regions of surgical resection specimen in non-radiated patients

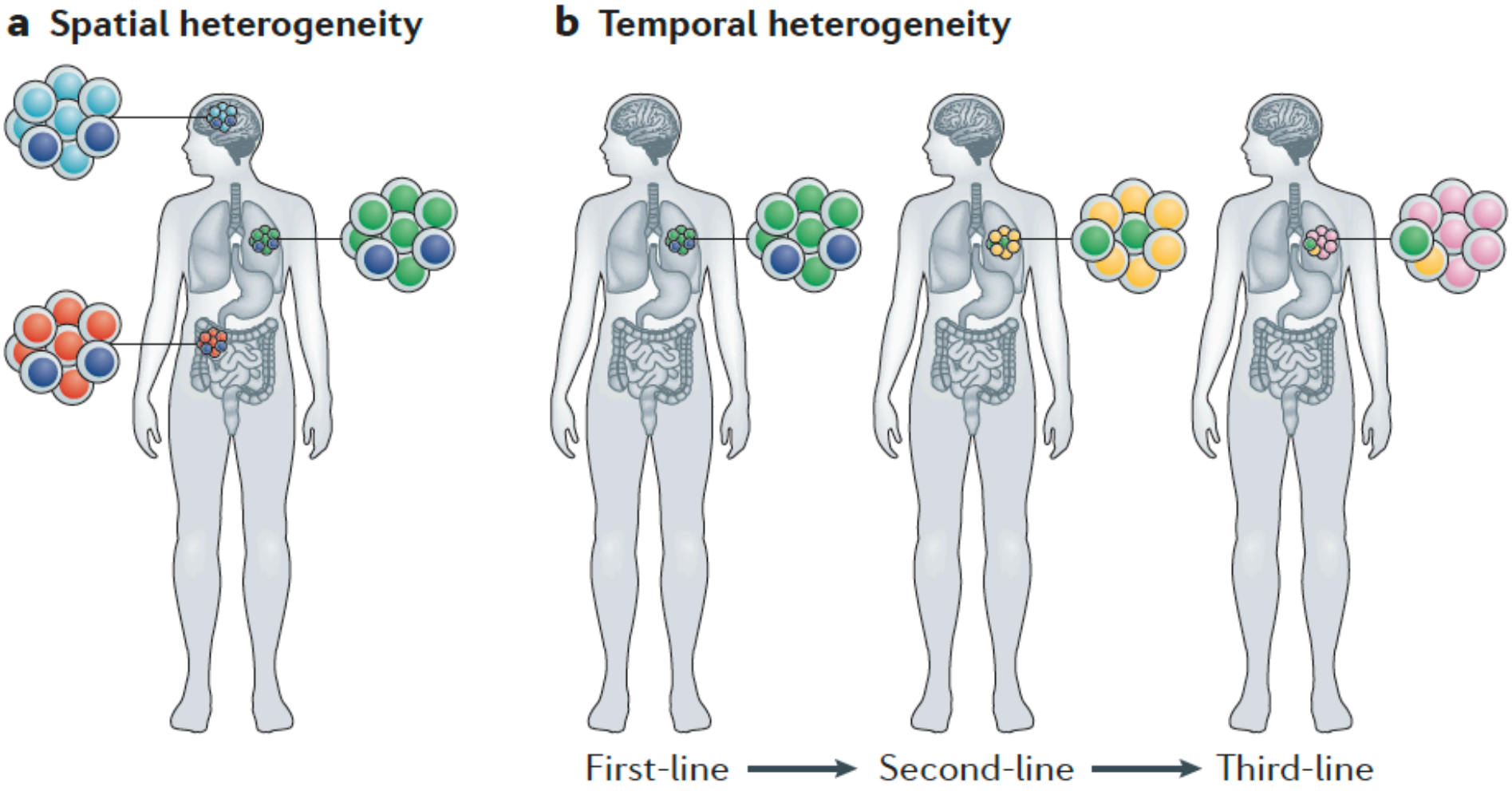
2. What are the differences and similarities between WD and DD components of a mixed WD/DD tumor?
(A) Compare sample regions of surgical resection specimen in non-radiated patients

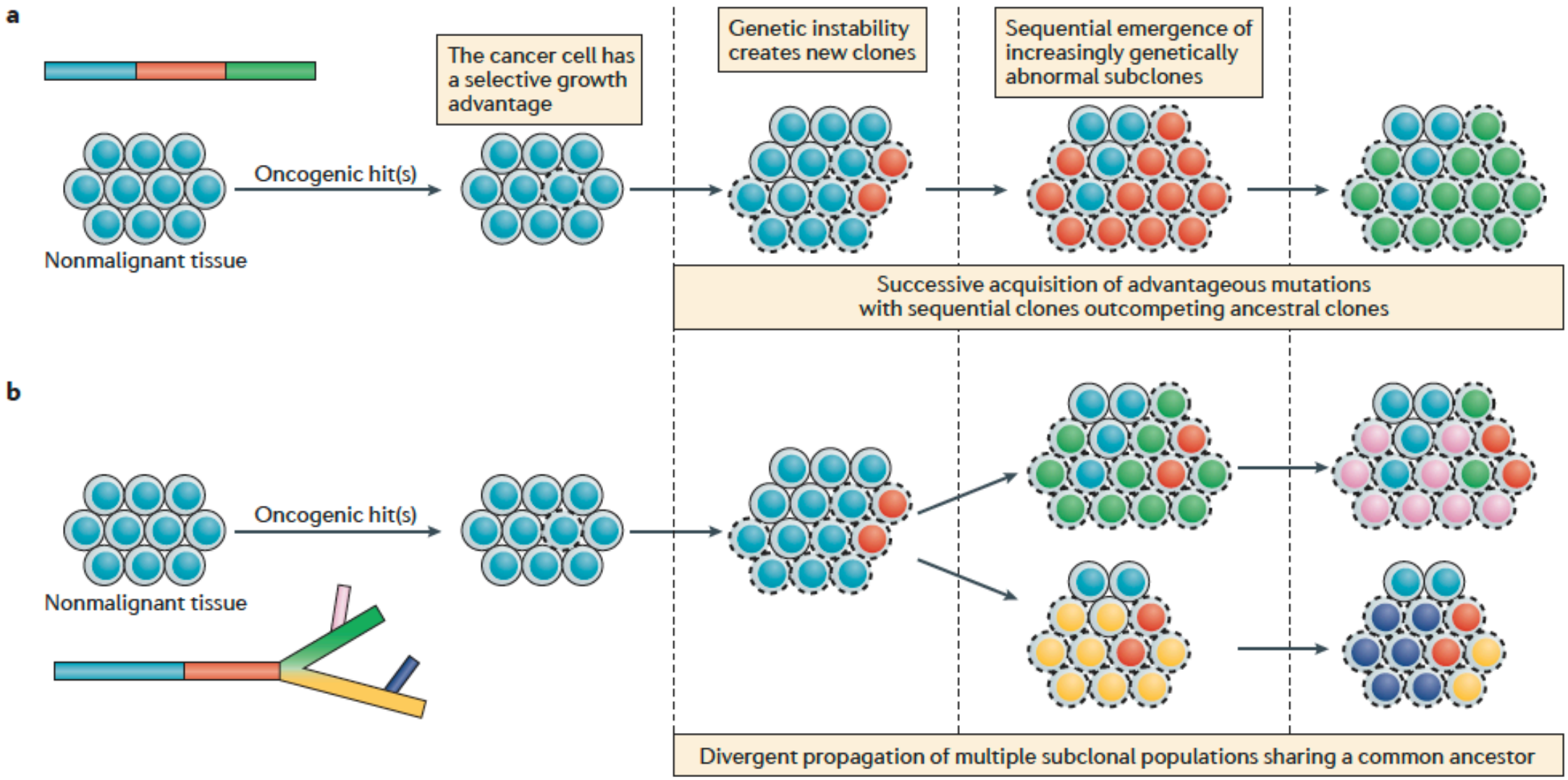
3. Do WD in a WD-only LPS differ from WD in a mixed WD/DD LPS?
(A, C) Compare banked primary WD tumor from patients who had WD-only primary with WD-only first recurrence vs WD component of a mixed WD/DD first recurrence (I think this is cleaner than cross patient comparisons)

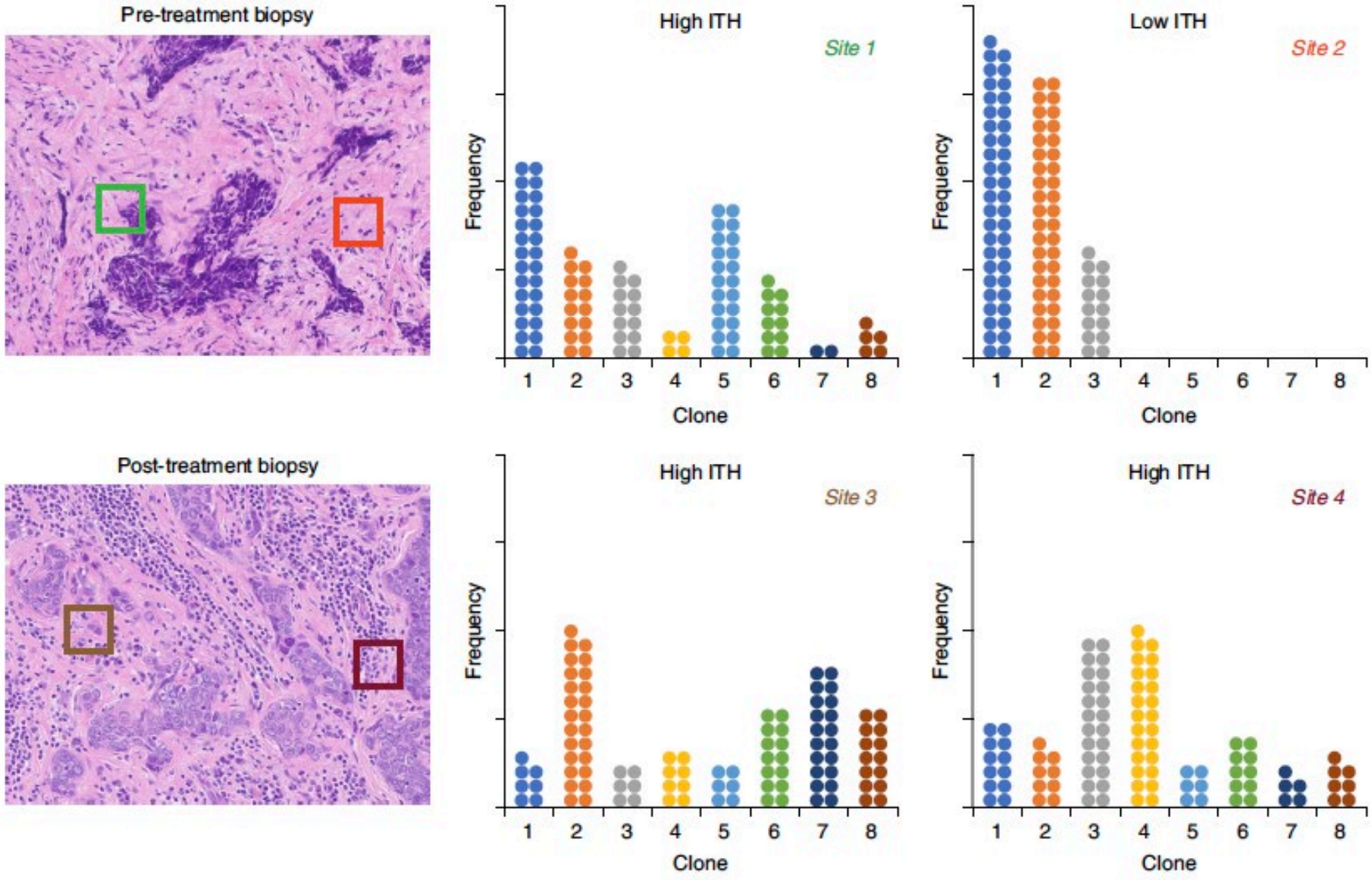
4. What changes are induced by preoperative RT, in the tumor and in the peripheral immune system? And timing of changes?
(D, E, B) Compare baseline, on treatment, and surgical timepoint tumor in RT group
(F, G, H) Compare baseline, on treatment, and surgical timepoint PBMCs and serum in RT group

5. How does LPS evolve from primary presentation to first recurrence? (overlaps some with question 3)
(A, C) Primary WD-only LPS to WD-only first recurrence (question 3)
(A, C) Primary WD-only LPS to WD and DD first recurrence (question 3)
(A, C) Primary WD and DD LPS to WD and DD first recurrence

Intratumoral Heterogeneity




a
The cancer cell has a selective growth advantage
Genetic instability creates new clones
Sequential emergence of increasingly genetically abnormal subclones
Oncogenic hit(s)
Nonmalignant tissue
Successive acquisition of advantageous mutations with sequential clones outcompeting ancestral clones
b
Oncogenic hit(s)
Nonmalignant tissue
Divergent propagation of multiple subclonal populations sharing a common ancestor


Pre-treatment biopsy
High ITH
Site 1
Low ITH
Site 2
Post-treatment biopsy
High ITH
Site 3
High ITH
Site 4
Frequency
Clone
1
2
3
4
5
6
7
8