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Working Group 2:

Transcriptional Patterns of Distinct Immune Landscapes

A more holistic approach

- We are increasingly thinking about host-specific factors (microbiome, metabolism) and transcriptional patters, but this still gives us a snapshot in time of these factors
- What about the evolution of these over time, and their temporal relationship to each other?
- Systems biology + multi-omics technologies (single cell sequencing, spatial resolution). Each of these technologies have their pros and cons
- What technologies do we need? For example, being able to tell from a single TCR sequence which Ag is being recognized (ongoing efforts). What do we do with this information and what's the translational applicability?

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TimIOs initiative

- Initiated during the SITC Sparkathon 2017 event
- Obstacle: Little is understood about distinct differences between responders and non-responders to checkpoint blockade
- Key issue: Compartmentalization of clinical and tissue-derived data

- Solution: A unified public/private consortium
- TimIOs was awarded $100,000 to address resistance to checkpoint blockade
- An honest broker to facilitate cross-institutional collaboration
- To develop a platform identifying fundamental differences between:
  - Durable vs. Transient responders
  - Elite responders vs. Rapid progressors

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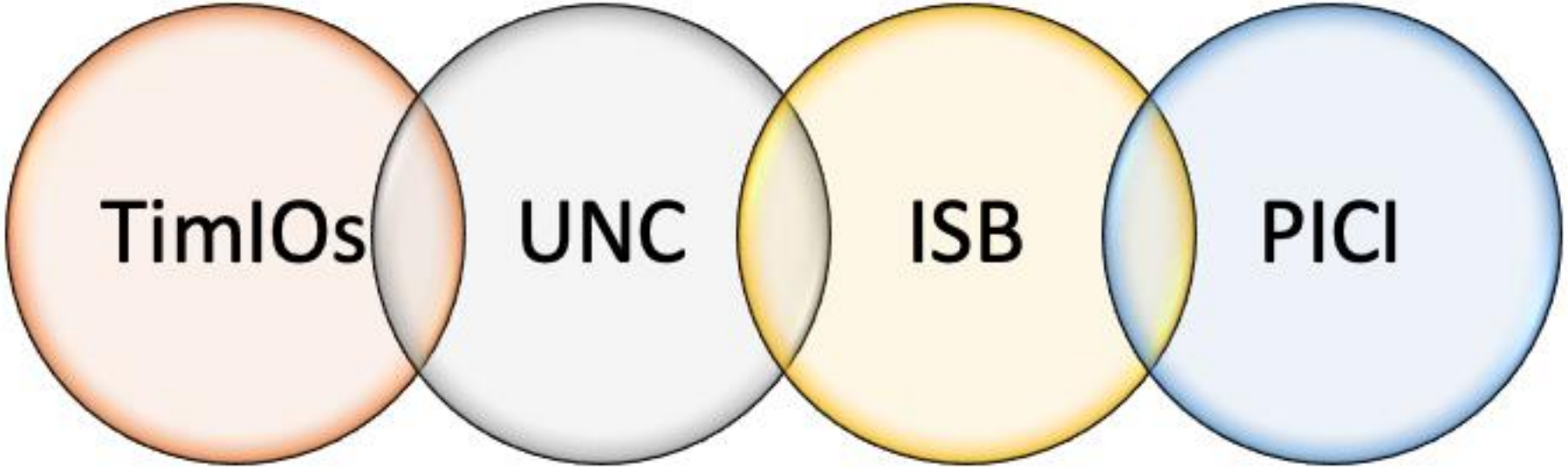
Development of larger TimIOs consortium



Researchers from Parker Institute of Cancer Immunotherapy (PICI), SITC-TimIOs, University of North Carolina (UNC), and the Institute of Systems Biology (ISB)

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Strategy

Correlate tumor profiling with patients' outcome to immunotherapy

1. *Transcriptomic data*

1. *Response/resistance*
2. *Survival*

1. *Checkpoint blockade*

Improve our understanding of the mechanisms underlying refractoriness to checkpoint blockade

Develop predictive/prognostic biomarkers to improve patients' selection and guide the design of more effective immunotherapies

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Tumoral Heterogeneity

- The big issue: Intra-patient, intra-tumoral, intra-metastatic heterogeneity
- Patterns of heterogeneity within a specific disease area
- Standardized instruments to describe heterogeneity (heterogeneity in our methodology is not helping)
- Which methodology can we use to best understand this heterogeneity?
- Which biopsy is most informative? (Pre-treatment vs. on treatment vs. on progression)
- What about the temporality, instead of just one snapshot over time. What's changing over time?
- What methodology do we have to evaluate change over time? (circulating tumor DNA, circulating tumor cells serial tumor biopsies, imaging analysis) Is our current methodology adequate to follow one disease over time? Host specific factors also (stress, metabolism, etc)
- What about new technologies/biosensors (google glass, apple watch): what can they measure, what applicability do they have to all the above? Easier monitoring over time

Applicability of Different Signatures

- How do all these immune signatures (MIP, IFN-g, Merck) correlate with each other?
- If we assay one sample with all of these, will they tell us the same thing? Will samples end up in the same ‘bucket’, hot vs. cold?
- Immune inflamed vs. non-inflamed- spatial resolution becomes important
- Prospective validation of immunoscore in diseases outside of CRC, and for primary vs. metastatic disease
  - ECOG would be a good platform to do this
- This is important when we consider how to move forward with these into the clinic

Single cell sequencing

- When is this methodology best used? Which questions is it best served to answer?
  - Is it more a discovery tool at this time? What about clinical applicability?
  - Different methodologies of single cell sequencing (ATAC-seq vs. RNA-seq vs. total-seq [might help resolve all the clustering we see])
  - In-situ assays (LCM)
  - More focus on structuring the questions up front, prospective validation of the experimental question

- Better ways of visualizing and communicating findings from different data sets
  - For example, samples are ‘clustered’ into different groups
  - This separation is in many ways artificial, putting samples/patients in different bins based on a single snapshot
  - What might a simpler approach be for presenting data from single cell sequencing?

Question 1

- What factors mediate a non-T-cell inflamed tumor microenvironment (tumor intrinsic vs. host)?
  - Differing definitions: how do we define ‘hot vs. cold’, ‘inflamed vs. non-inflamed’. Are all cold tumors the same? How does this impact our clinical decision making?
  - PTEN, beta-catenin, PARP, IFN-g, etc..
  - Organ-specific: factors that need to be considered? BBB, pancreas– location. What’s the role of the stroma?
  - Host specific: metabolism, microbiome
  - Tumor specific: antigenicity, TMB, etc
  - Immune specific: Immune infiltrate/peripheral immune: myeloid, Treg, macrophage
  - Inflamed vs. non-inflamed. What do we mean? Is this a transcription based definition (MIP, Merck, Gajewski, IFN-g). Immune contexture signature may define outcomes.

Question 2

- What are the transcriptional alterations in patients with primary vs. secondary resistance to immunotherapy?
  - Are they different?
  - What about pts with CR vs. hyper-progressors. How are they different?
  - Not just transcriptional alterations- also loss of ploidy, chromosomal instability

- Which transcriptional alterations are mediating T cell exclusion within a treatment naïve tumor?
  - How do we define a cold tumor? May need to look at alterations across different tumor types
  - Are different tumors cold for different reasons?
  - Are the alterations different across different tumors? Are there any that are prevalent across a certain tumor type (for example an oncogenic pathway)
  - Which cold tumor can be remodeled to a hot tumor?
  - Are they distributed evenly across different tumor types (probably not). There is likely heterogeneity in transcriptional alterations

Question 3

- Should we be directly targeting the alterations in signaling pathways or changing the immune infiltrate by targeting immune cells directly?
  - Understanding the mechanism of the tumor's 'coldness' is key
  - Depends on the alteration. If oncogenic driver, need to address that directly since it may be mediating immune exclusion. If it's that the tumor can't present antigens, for example, then targeting the immune cells directly may be more useful
    - Inter-play between the two (for example, rationale combination strategies that address each of these)
  - For example, BRAF/MEKi+anti-PD1 in advanced melanoma to increase CD8+TIL
  - Does the dose of a drug differ if we are targeting the tumor vs. the immune system (for example STAT3)

Question 4

- What are the best approaches to model the non-inflamed TME and to elucidate new pathways/immunological mechanisms of T cell exclusion in humans?
  - Again, how are we defining non-inflamed TME? What are the thresholds/cut-offs?
  - Metabolism is an interesting angle to look at remodeling the TME- for example decreasing oxidative metabolism of correlates with improved TIL function and improved responses to anti-PD1. What about transcriptional signatures over time?
  - Microbiome (FMT transplant)
  - Example of where single cell sequencing might be of high utility
  - Multiplex channels to measure transcriptional alterations

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Issues that need to be addressed

- Temporality is critical in understanding the development of the tumor and the tumor-specific immune response and mechanisms of escape/resistance
- Remember that association is not causality: just having mutations or a certain transcriptional alteration does not establish causality (esophageal cancer, melanoma)
  - Knowing when the host ellicits a response vs. not is key
  - Importance of early detection and prevention
- What is the interplay between transcriptional patterns and other host factors (microbiome, metabolism, etc)
- Interplay between cold tumor and immune system- which came first? Is the tumor cold because the immune system didn't enter, or was it eradicated?
- What questions are best answered with singe cell vs. more global profiling?
- What about spatial resolution? How to decide which methodology best suits each question? Clinical vs. research methodology.
- A lot of this data is siloed with pharma- TimIOs consortium, a shared bank of brightfield images is an ongoing effort
- Generation of a hierarchical structure evaluating the significance of these different factors

Issues that need to be addressed

- ‘Master regulators’ might be helpful in terms of classification of tumors. For example, PD-L1 expression predictive in certain (but not all) disease. Often disease specific. Are there others? How might they be actionable down the line?
- Interdisciplinary setting key: computational biology, clinicians, pathologists, etc. How to incentivize key stakeholders?
- Describing findings vs. prospectively asking the question so we can come one step closer to validating transcriptional signatures

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Output Plan

- Position paper:
  - What to include in future studies of IMT- standardized ‘immune marker’ across studies. For example, be more specific about TIL infiltration within the tumor
  - Need an immunopathologist
  - What to consider. For example, how do cells get in and out (vasculature, lymphatics), unified way of prospectively measuring the immune cell repertoire within tumor types
  - Novel approaches that need further evidence for consideration. For example, metabolism

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