Developing a Program in T-cell Therapy for Soft Tissue Sarcoma

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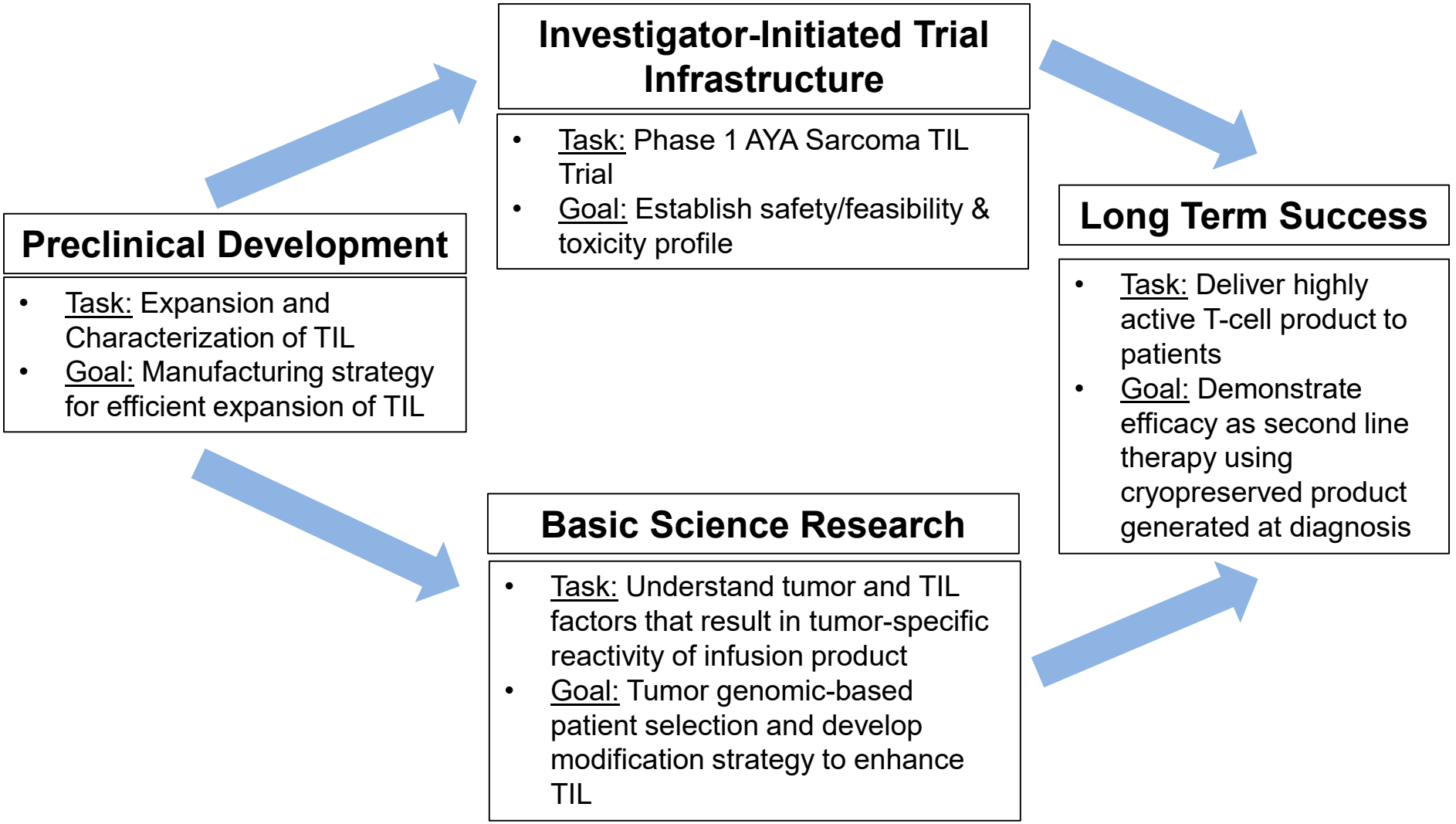

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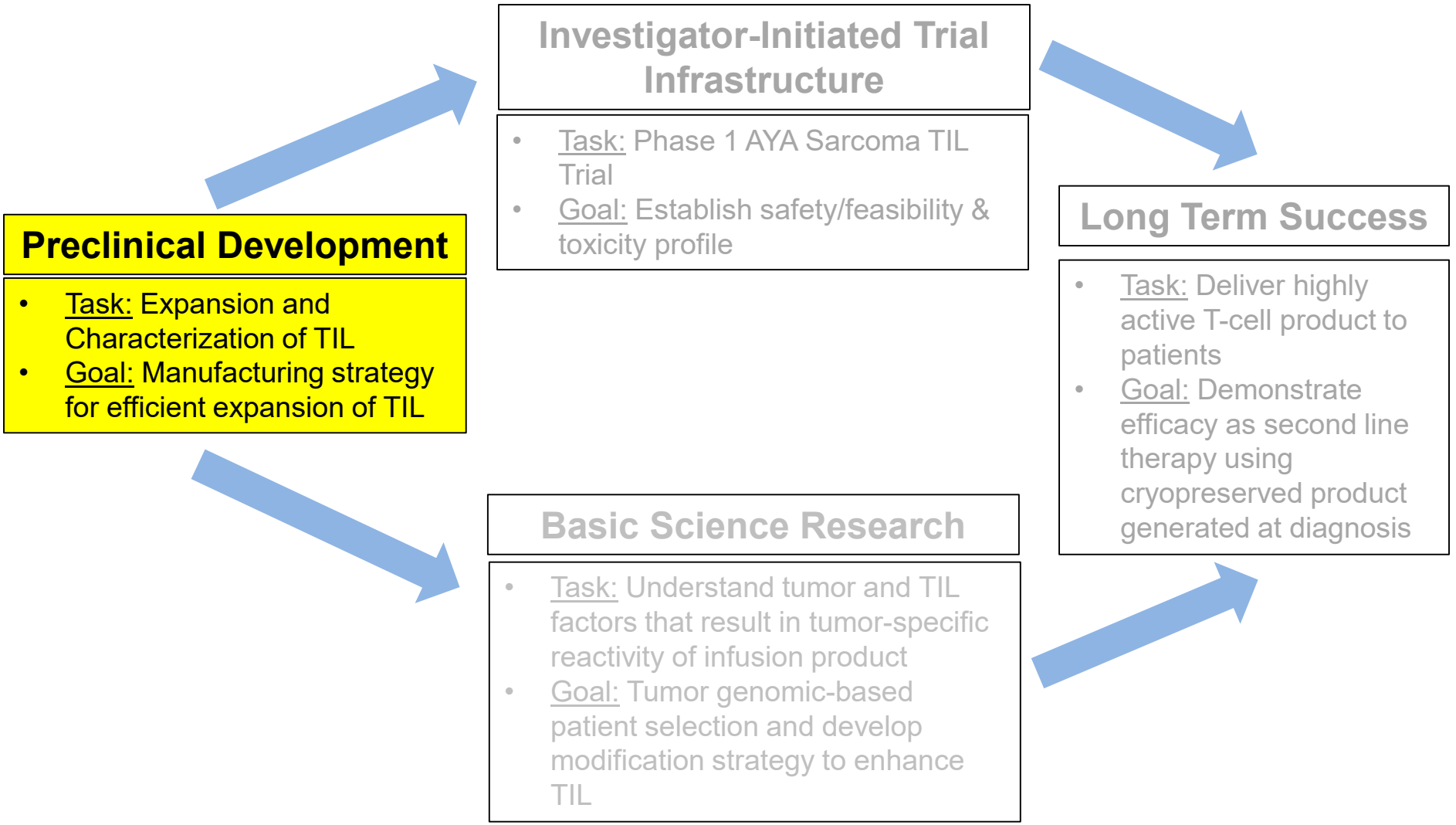
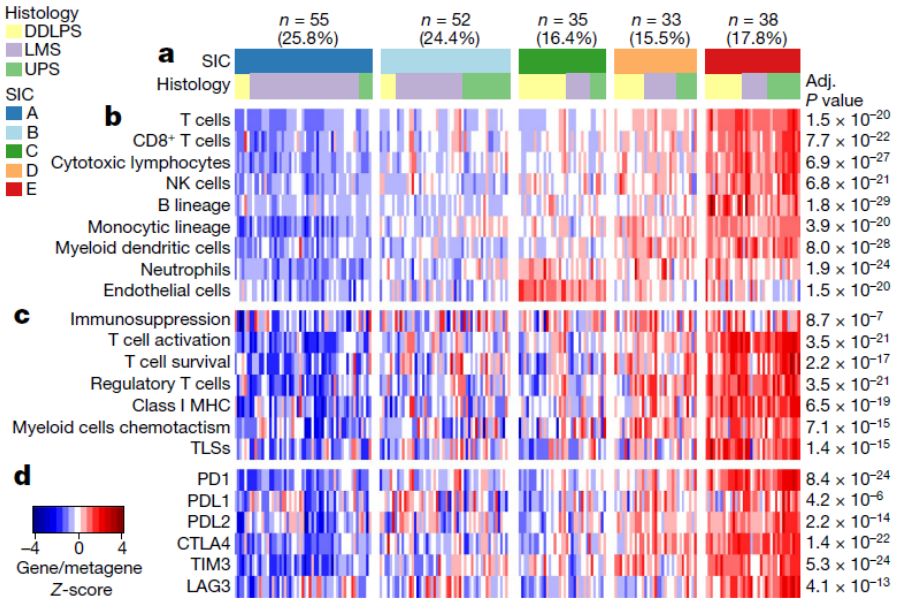
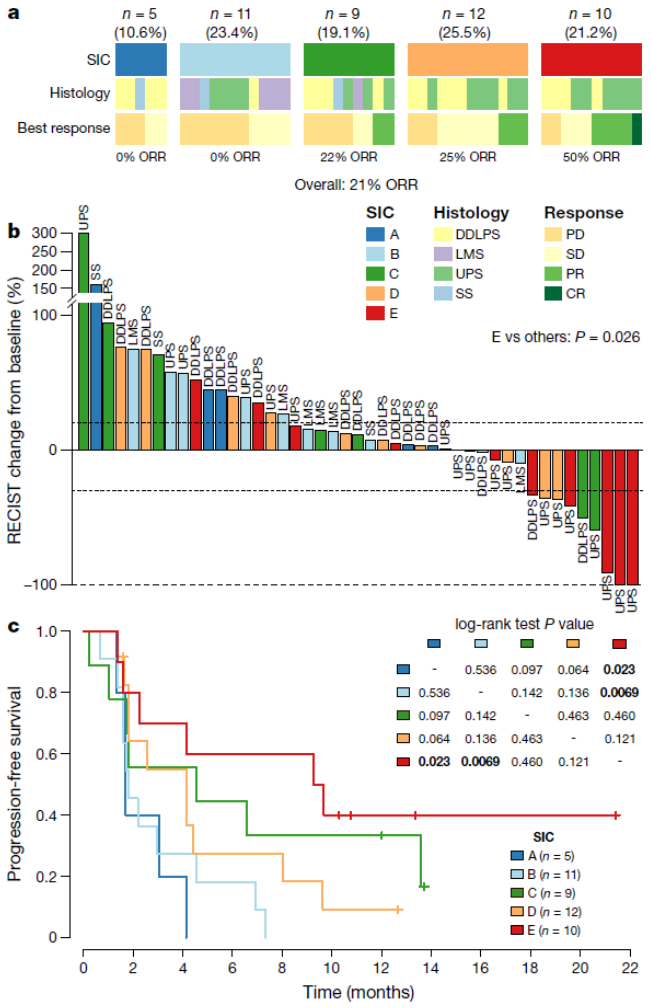


Gene Expression Data Categorize Immune Phenotype and Predict Outcome in Sarcoma

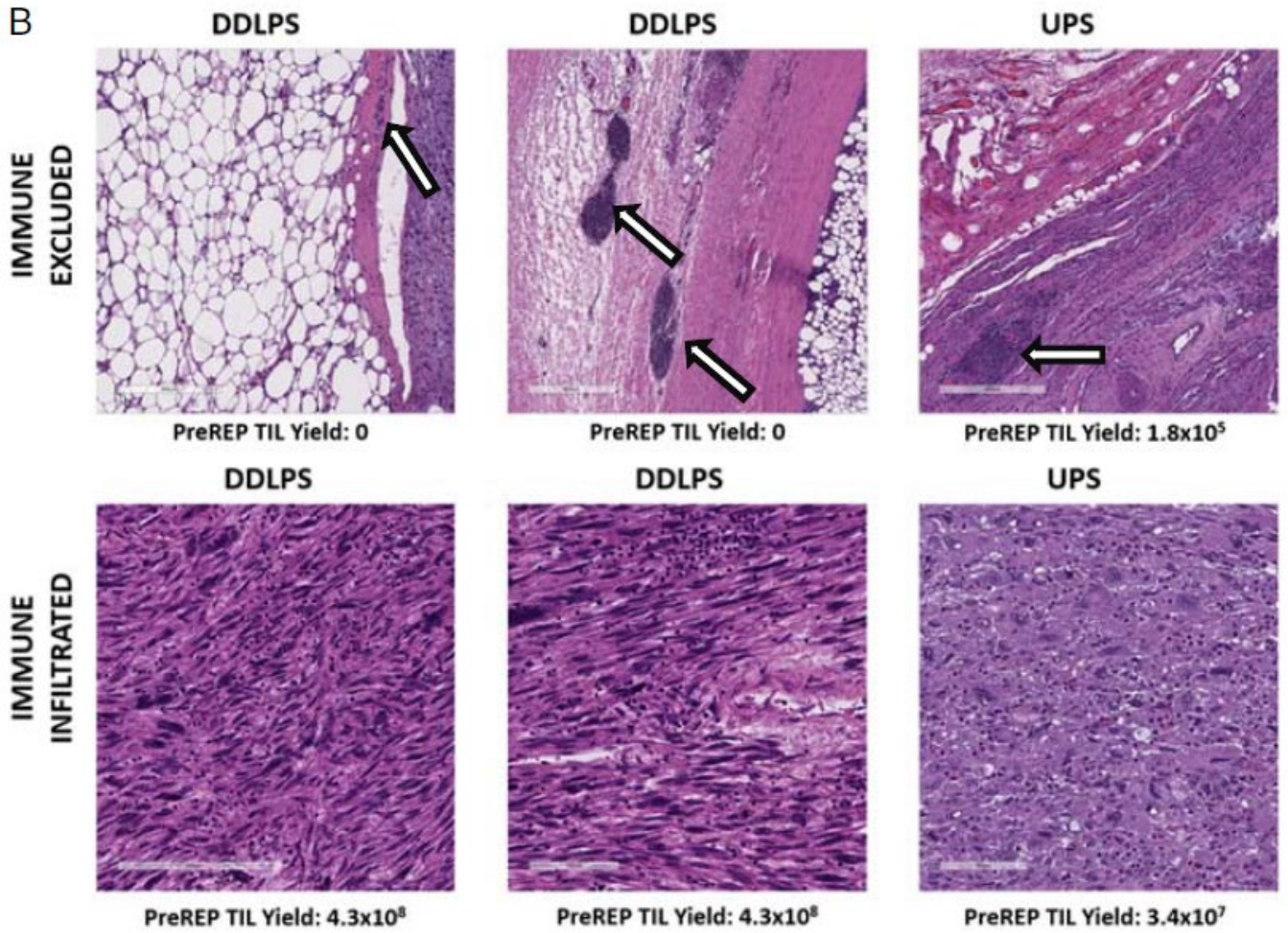




- Four publicly available datasets:
  - TCGA SARC, Gene Expression Omnibus accessions GSE21050, GSE21122 and GSE30929
- Microenvironment Cell Populations-counter (MCP-counter) method
  - *Becht, Genome Biology, 2016*
- 608 tumors analyzed to create 5 unique Sarcoma Immune Classes (SIC)

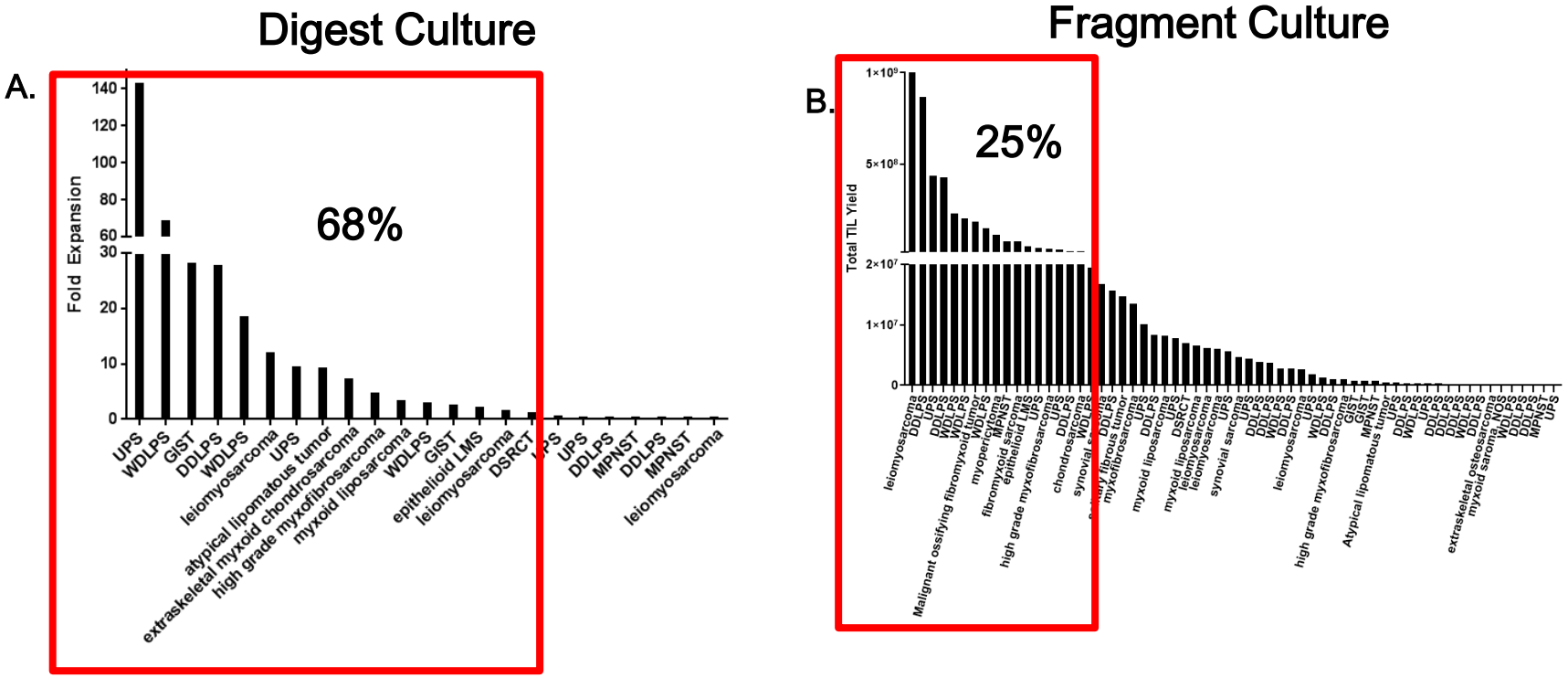
Petitprez, Nature, 2020

Lymphocyte infiltration pattern predicts TIL expansion from fresh tumor



Mullinax, J Immunotherapy 2021

TIL Culture From Fresh Tumor Digest Increases Likelihood of Successful Expansion



Mullinax, J Immunotherapy 2021

Preclinical Manufacturing Data Summary

Key Results:

- TIL can be expanded from primary sarcoma specimens
- Cultured TIL REP to clinically relevant degree
- Digest method yields highest probability of generating TIL infusion product (Mullinax et. al., J Immunotherapy 2021)

Deliverable:

- Successful manufacturing approach offers opportunity to test the feasibility of ACT for STS
  - FDA IND # 18646
  - Patent # PCT/US22/24804, 10615-301WO1
  - Phase 1 clinical trial protocol

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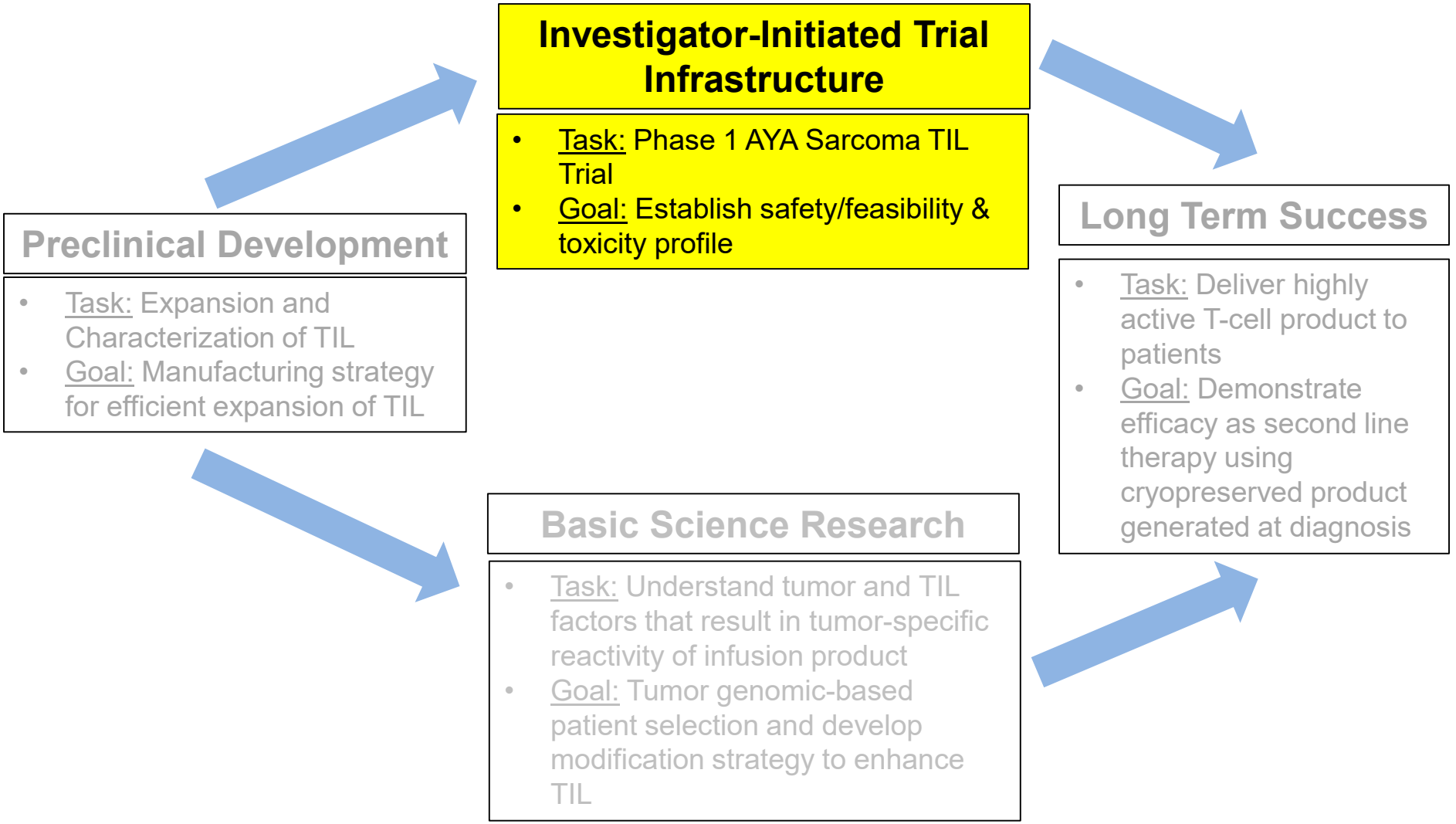
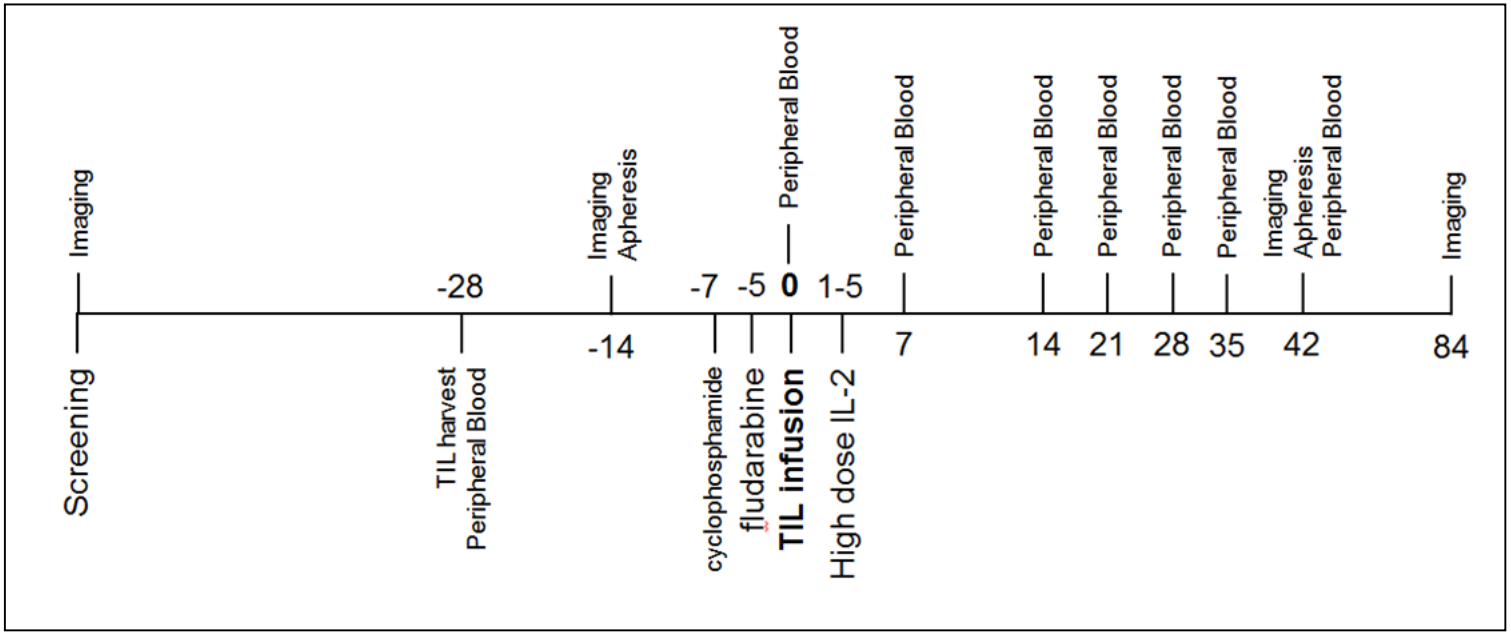


MCC19837: “AYA Sarcoma TIL Trial”

- Metastatic STS, failure >1 line systemic therapy, age 18-40
- Treatment Plan:
  - New production process: preREP TIL product from tumor digest
    - REP unchanged
  - IL-2 post-TIL infusion: high dose schedule (600,000 IU/kg q8h) with standard hold and discontinue parameters utilized on Cell Therapy inpatient service



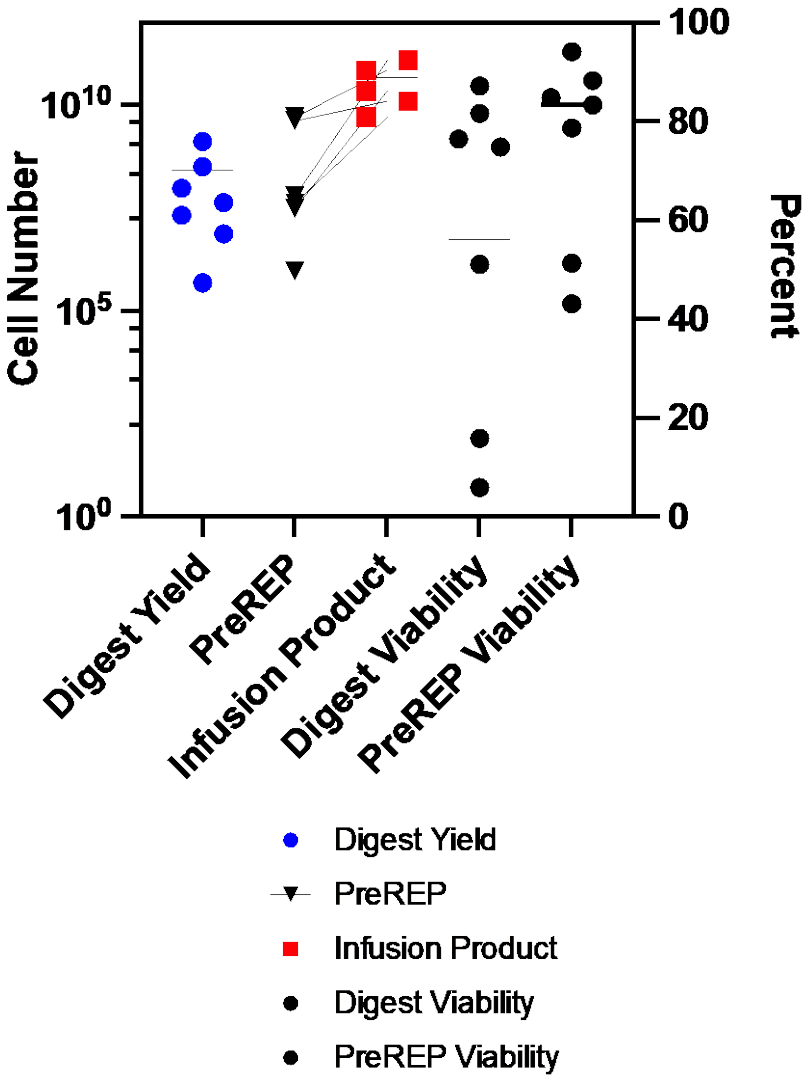
Enrollment Demographic Summary

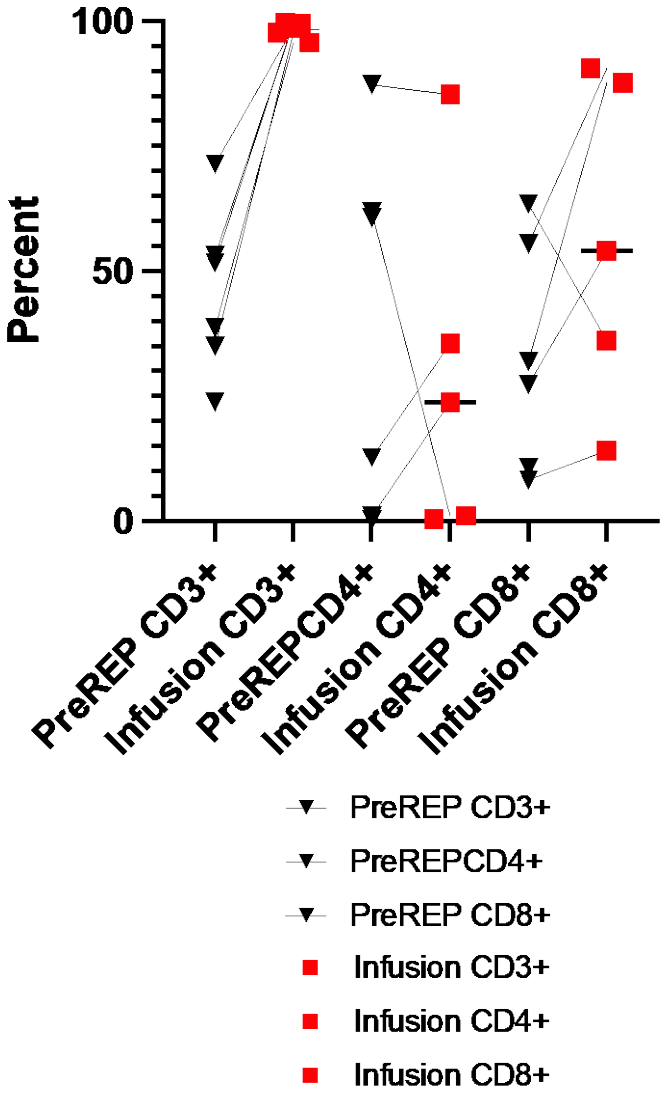
Total enrollment	**N=9**
Gender	7/9 (78%) male
Age	27.4 (24-38.5)
Prior lines of therapy	2 (1-5)
Sarcoma subtype diagnosis	Myxoid liposarcoma (2) Clear cell sarcoma Synovial sarcoma Desmoplastic small round cell tumor Ewing sarcoma Leiomyosarcoma Extraskeletal mesenchymal chondrosarcoma Osteosarcoma
SAE, treatment related	3/5 (60%) • 2 Cy/Flu: G3 DAH, G4 bone marrow hypoplasia • 1 IL-2 related: G3 hypotension
Expansion success	5/7 (71%) • 2 screen fail (EMS, LMS) • 2 non-expansion (osteosarcoma, Ewing)
Infusion product	$2.16x10^{10}$ ($5x10^{9}$-$6.87x10^{10}$)
IL-2 doses received	4 (1-7)

Primary Endpoints:

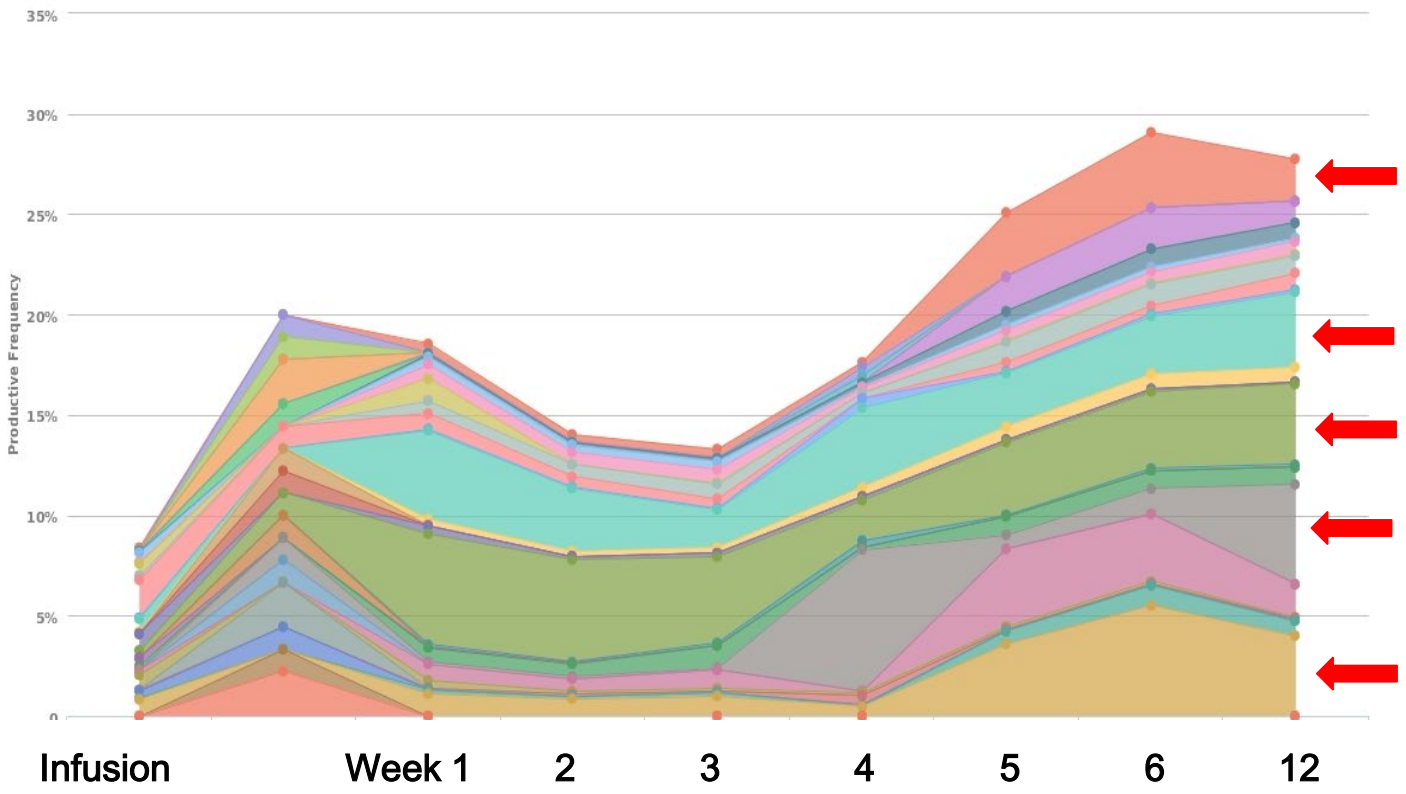
- Safety: No more than 3 patients with >G4 non hematologic AE
- Feasibility: Expansion of infusion product from 33% of patients

High quality infusion products were generated from resected metastatic sites





Infused TIL persist and T-cell clones preferentially expand over time



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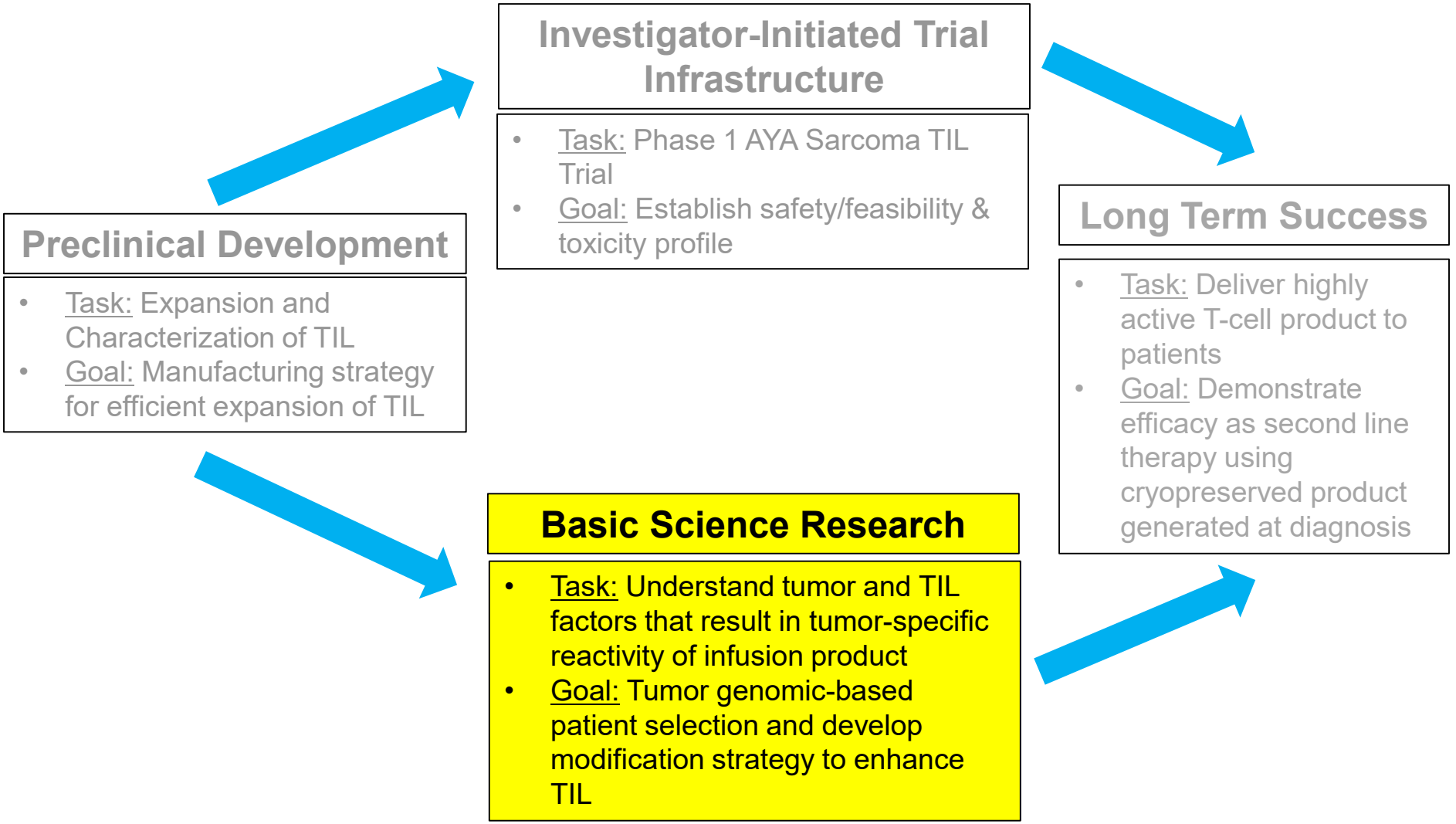

How do we define success for autologous T-cell therapy in sarcoma?

- Optimal patient selection
  - Identify tumor factors that predict expansion of reactive TIL
    - WES analysis using AVATAR dataset
  - Decrease burden of disease
    - TIL harvest before SOC chemotherapy
    - Cryopreserve with infusion post-failure
      - Allows for modification while receiving treatment
      - Significantly decreased logistical burden
- Optimal T-cell product
  - Identify T-cell subsets with tumor-specific activity
  - Strategy to modify T-cell product during manufacturing to elicit optimal phenotype

Leveraging Moffitt AVATAR Dataset for Preliminary Data

MCC patients with expanded TIL product n=106

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ORIEN AVATAR

↓

Patients with WES/RNASeq available n=24

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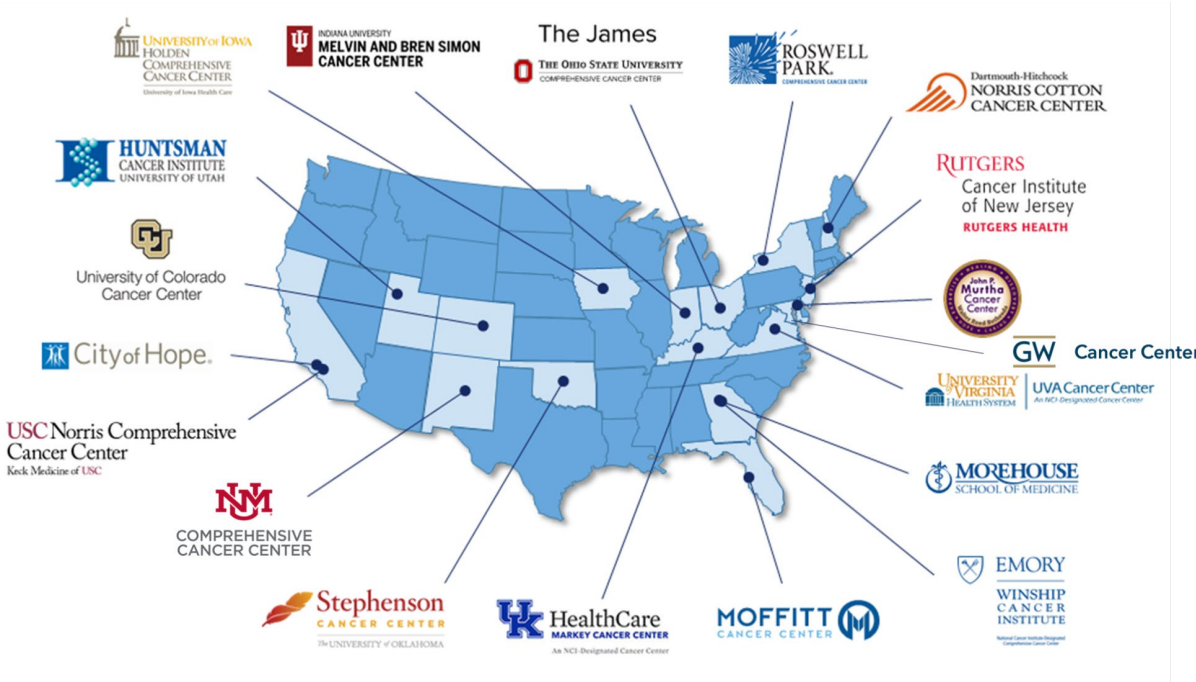
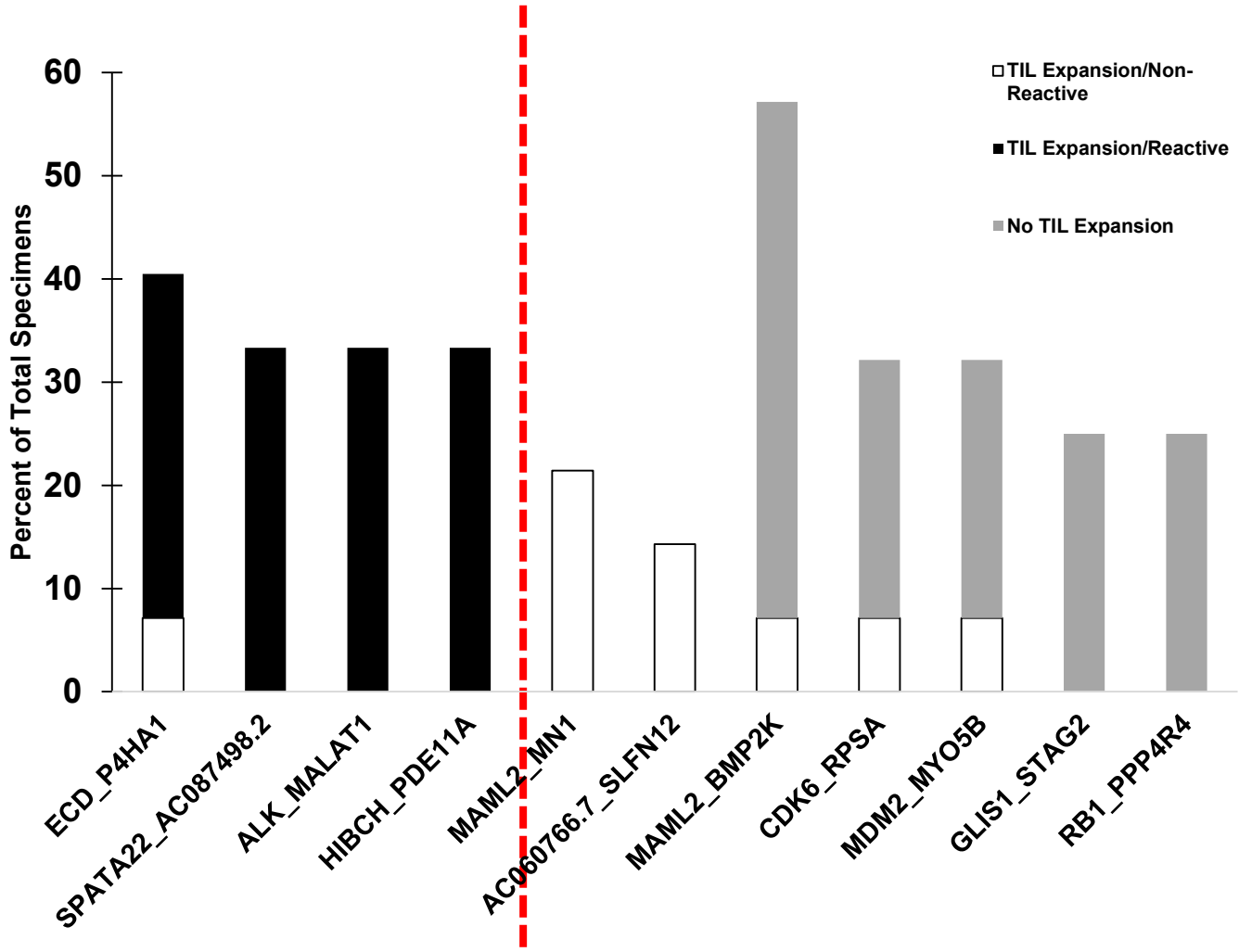
MCC Bioinformatics Pipeline: CNV, Gene Expression, Pathway Analysis

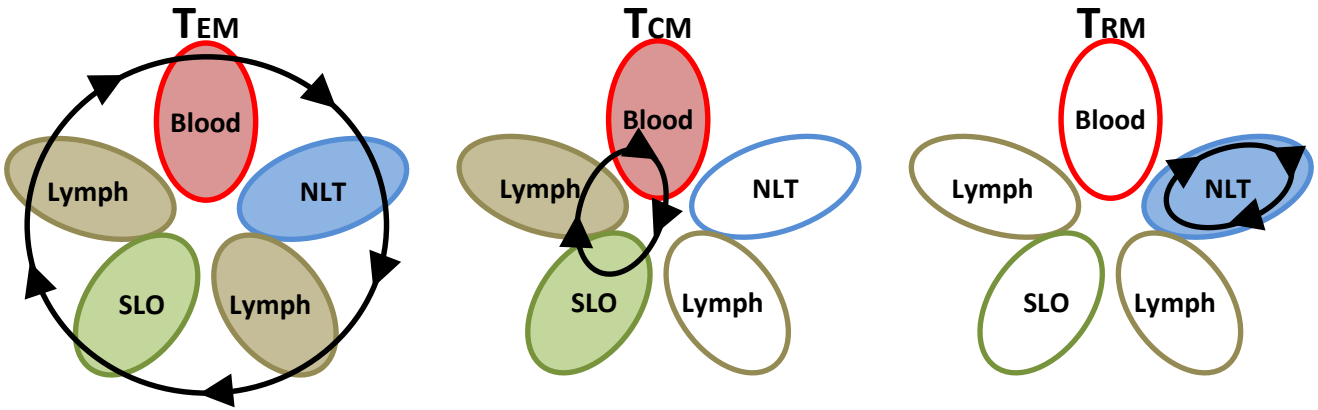


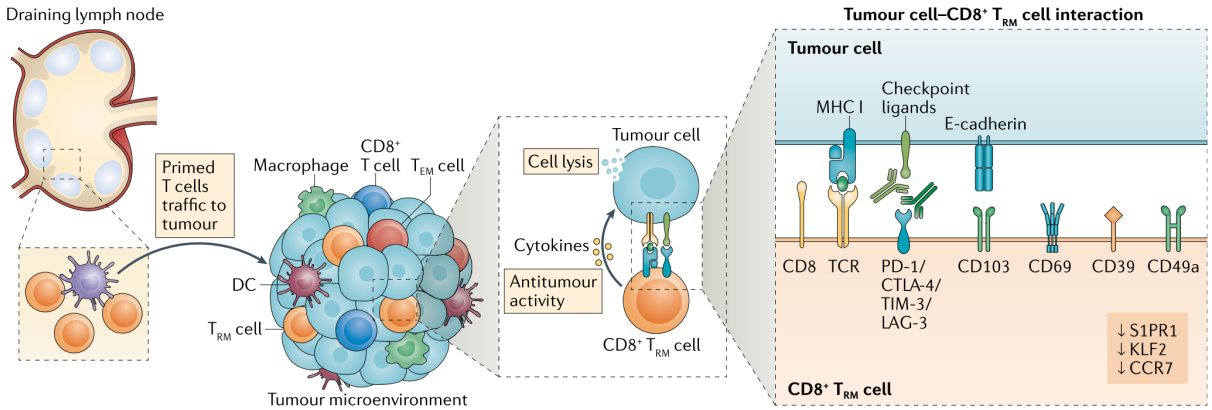
Unique Gene Fusions are Associated with Optimal ACT Phenotype



Chia-Ho Cheng, Ling Cen

Expanded T-cells from Fresh Tumor Include Multiple Heterogeneous Subpopulations





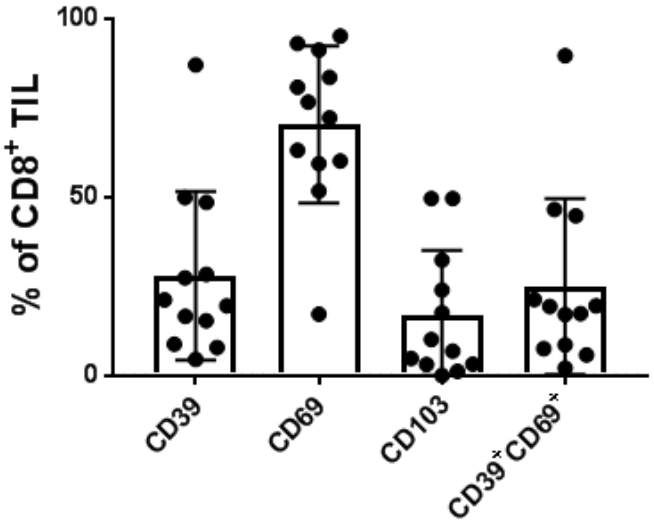
- Most literature focuses on carcinomas
- **We hypothesize** that optimal TIL phenotype will have a variance from the canonical markers shown in epithelial malignancies

Byrne, Nat Rev Clin Onc, 2020

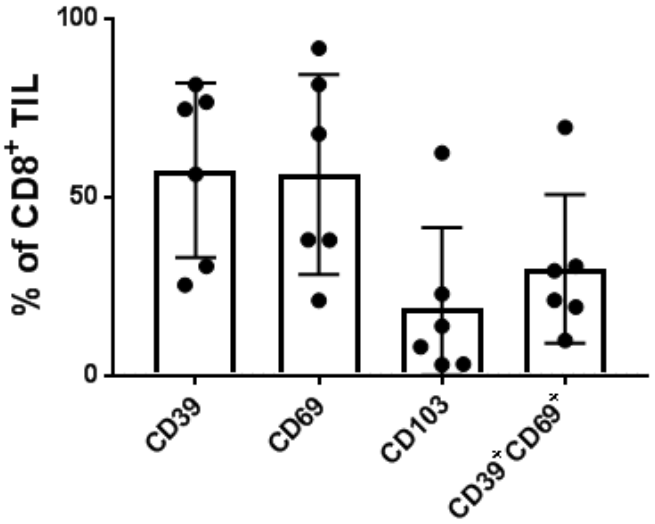
Multiple high impact publications describing function of T_{rm} in recent years

Author	Journal	Year	Phenotypic Marker	Key Conclusions
Ganesan…Ottensmeier	Nat Immunology	2017	CD103+ CD69+CD49a	Associated with residency program and higher OS in NSCLC
Duhen…Weinberg	Nature Communications	2018	CD39+CD103+	"Double positive" have higher anti-tumor activity and better OS in HNSCC
Lowery…Rosenberg	Science	2022	CD39-CD69-	"Double negative" TIL in infusion product associated with high CR rate
Anadon…Conejo-Garcia	Cancer Cell	2022	CD69+CD103+	Small percentage (3%) of TIL maintain "waves of effector TRM-like cells"
Crowl… Goldrath	Nat Immunology	2022	CD69+CD103+	Differential expression across normal tissue lineage

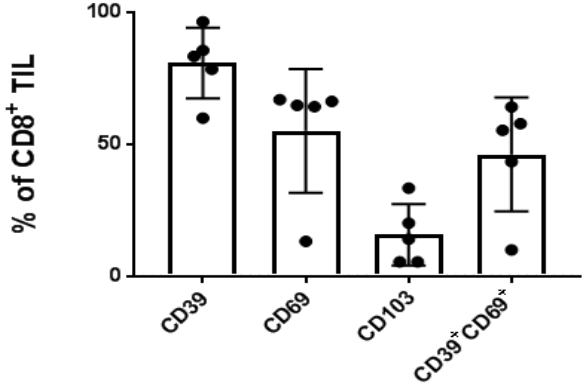
T-cell phenotype across phases of ACT in soft tissue sarcoma



Digest

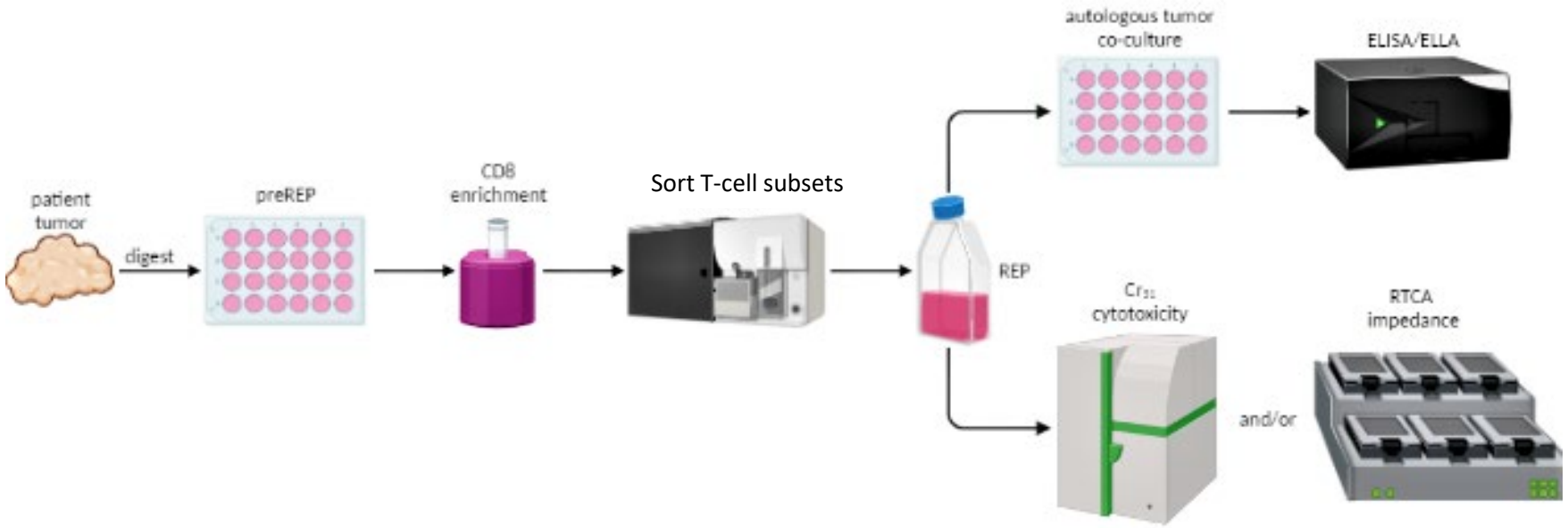


Pre-REP



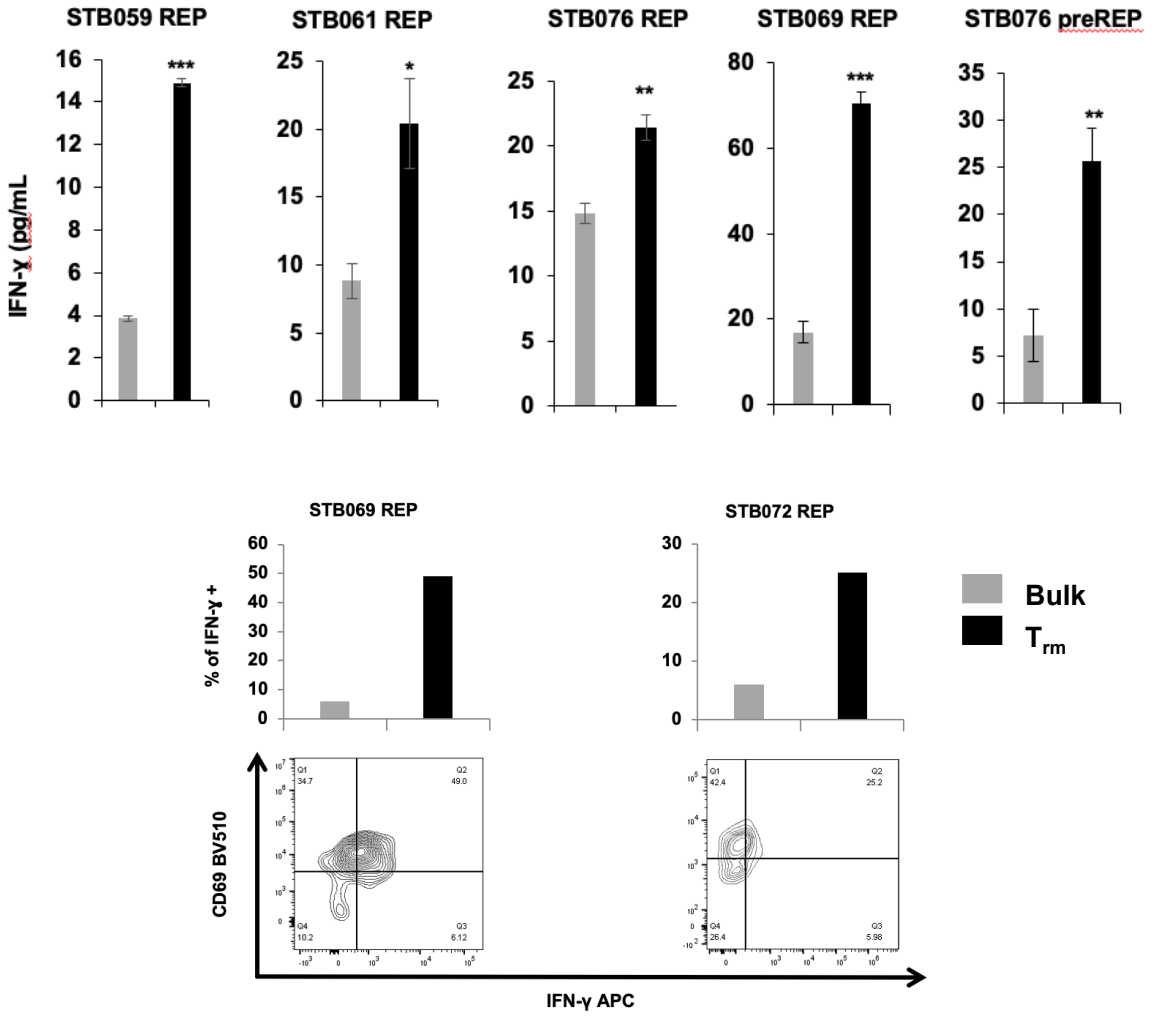
Post-REP

Functional analysis of T_{rm} Derived from STS



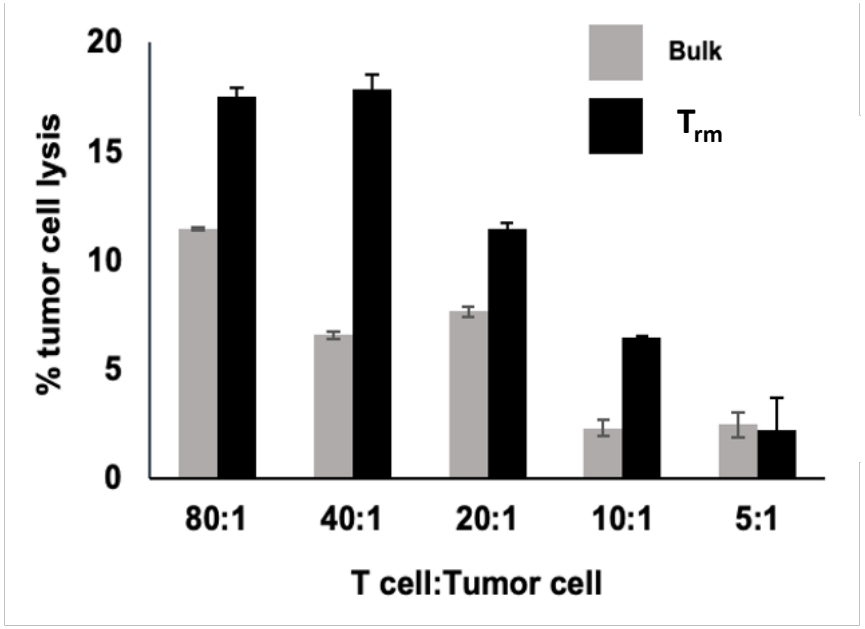
J. Hensel, A. Alfaro

T_{rm} Sorted TIL Have Enhanced Tumor-Specific Activity: Autologous tumor co-culture

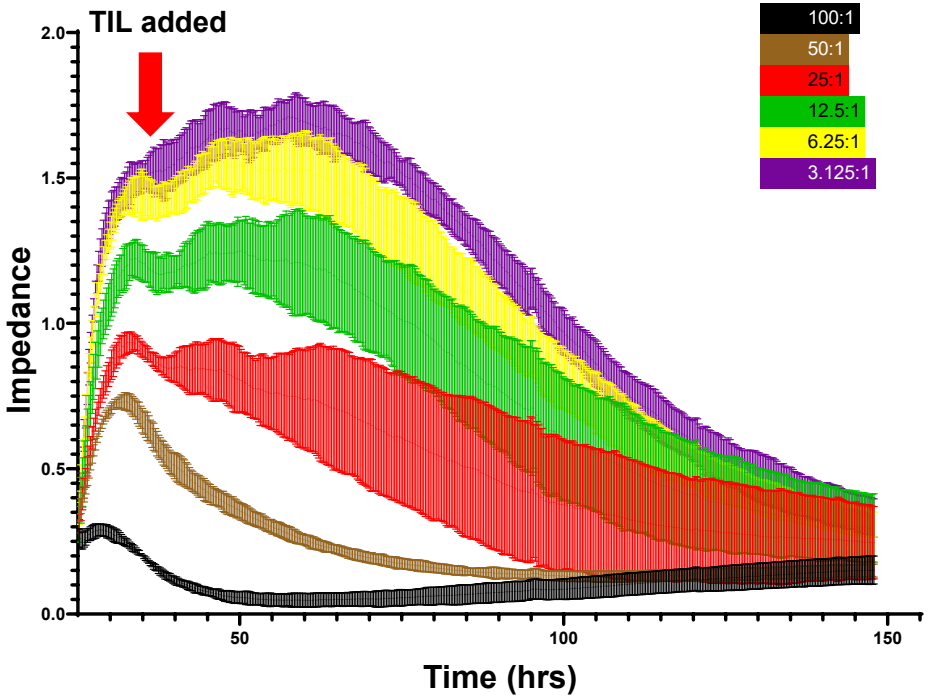


T_{rm} Sorted TIL Have Enhanced Cytotoxicity to Autologous Tumor Cell Lines

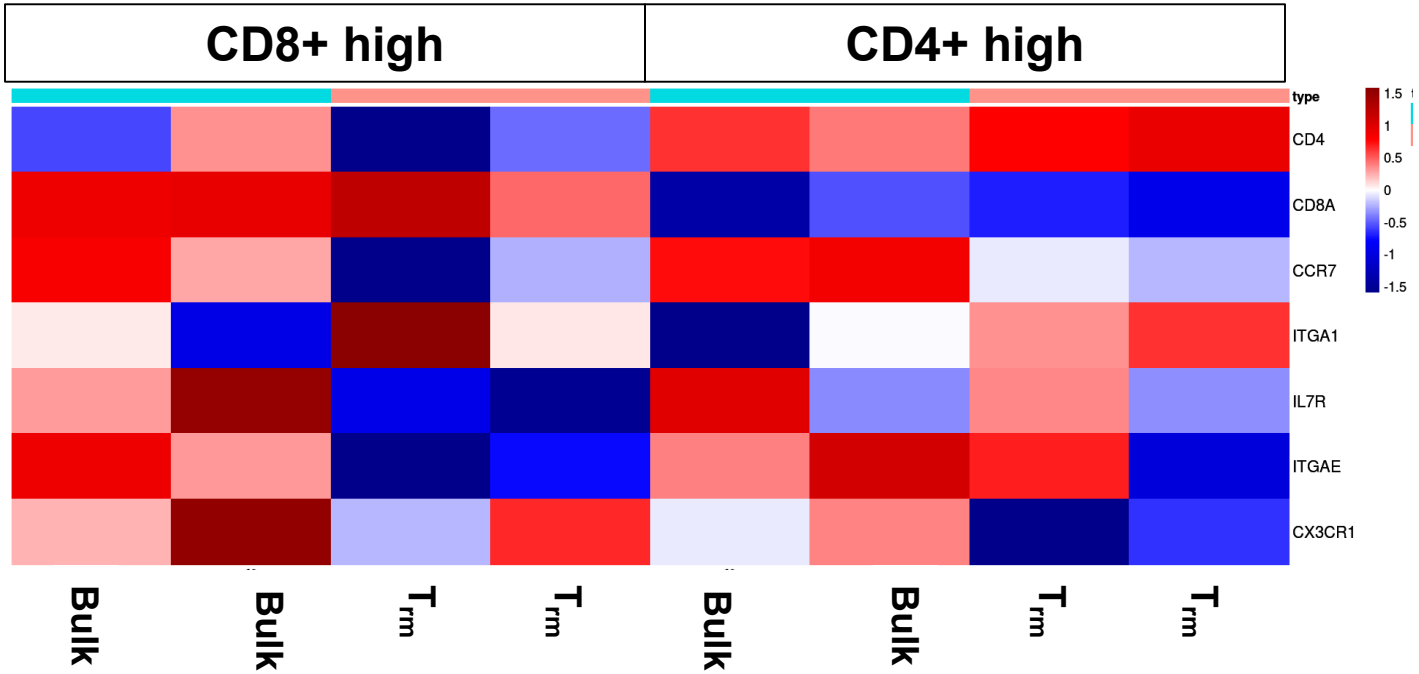
Cr51 Assay



Agilent xCelligence RTCA



RNASeq of Sorted T_{rm} populations reveals early signal of DEGs



Key preliminary findings:

- CD49a (ITGA1) high, supporting these cells are Trm-like
- CCR7 low, suggesting this population is these cell are not part of the central memory compartment
- CX3CR1 low (except for STB076), suggesting not Tem
- CD127 low (IL7R), which is more Temra-like (terminal effector memory T)

Timothy Shaw

Next Step:
ACT Modeling in humanized murine model

- 121 tumor samples acquired from lab (MCC18609)
  - Cohort of samples with matched
    - postREP TIL
    - PBMC (to generate EBV transformed B-cells as APC)
    - Tumor cell line – provides for RTCA, co-culture to document in vitro activity
    - PDX strain established

- Using a humanized murine tumor model (NOG-hIL-2) with matched human tumor-TIL we can effectively study the specific function of T-cell subsets from expanded TIL

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