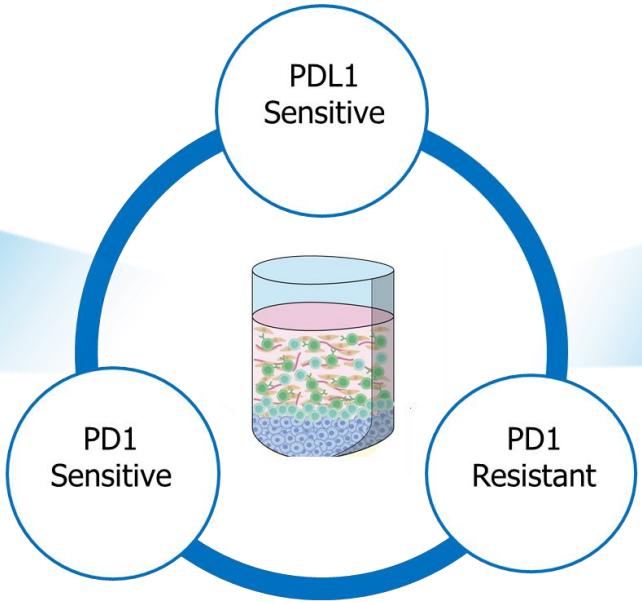
IMMUNE 3D®

A New Dimension to 3D Co-Cultures

IMMUNE 3D®

IMMUNE INFILTRATION



IMMUNE MIGRATION

SIMPLE

COST-EFFECTIVE

PATENTED

Challenge in IO Drug Development

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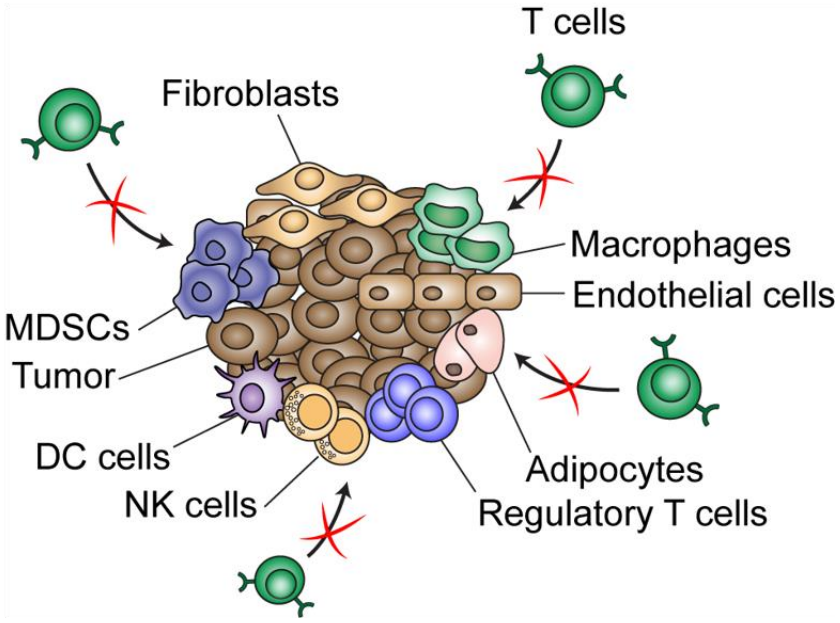
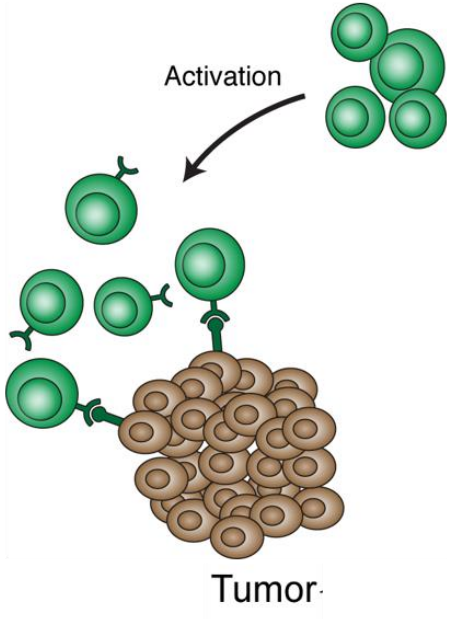
Need for efficient and cost-effective translational systems to study TME factors that impede T cell migration and infiltration

Problem



Goal

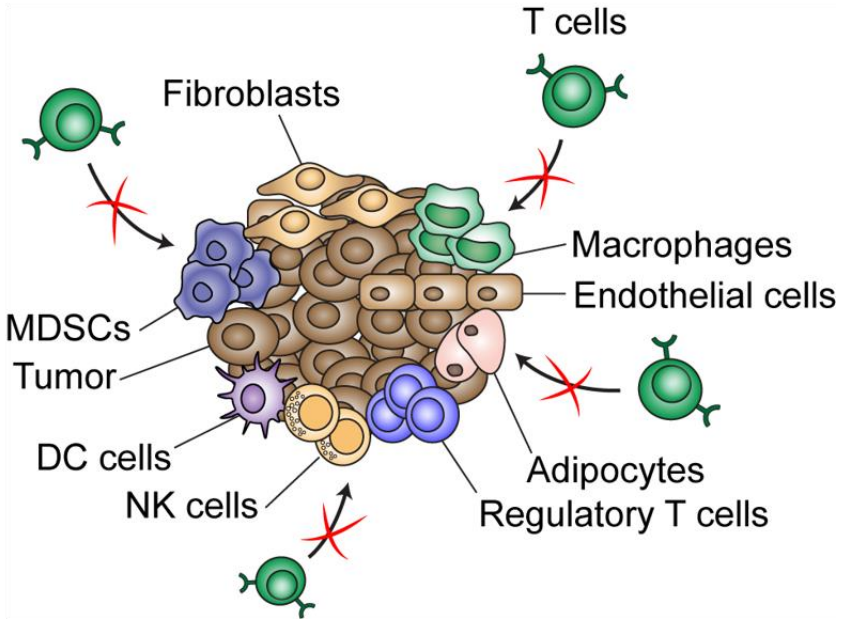


Unique and Proprietary Platform

IMMUNE 3D®

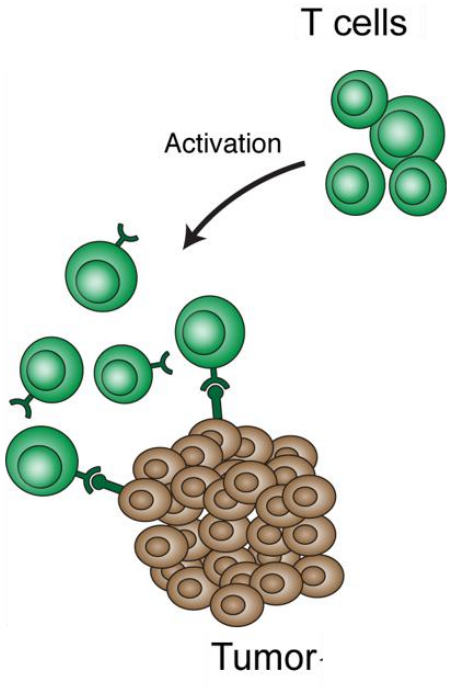
To create the tumor enviroments for effective drug screening

Problem



IMMUNE 3D™

Goal



Overview and Advantages

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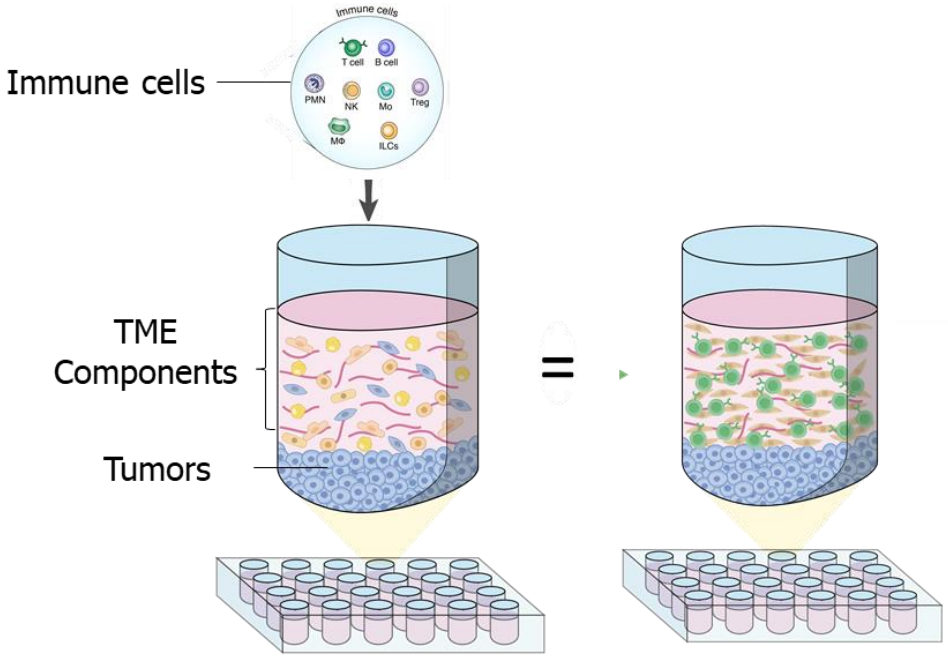
TME + Human cell line platforms = Tumor Immune Environment



- **SIMPLE DESIGN**
  Immune-Tumor Interactions
- **PATENTED SYSTEM**
  Migration and Infiltration
- **BROAD TUMOR**
  Applications and Readouts
- **NOT SPHEROID**
  Dependent

TIME = Tumor Immune-Microenvironment

Advantages

IMMUNE 3D®

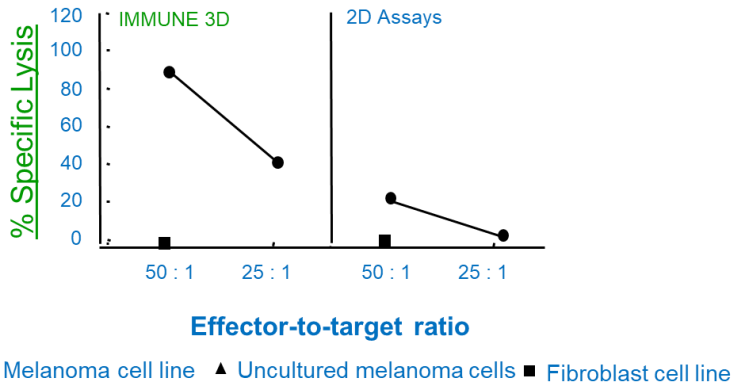
Greater Specificity
Stringent system to evaluate antigen presentation

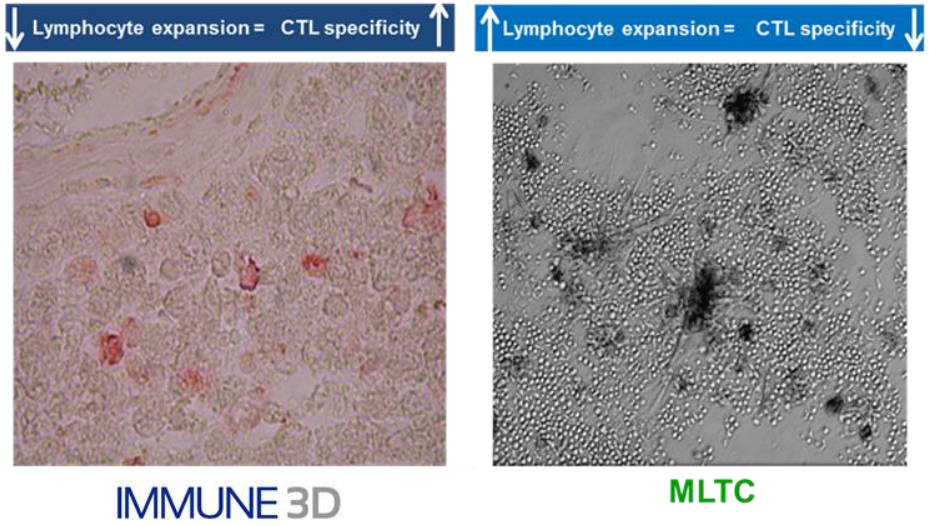
Immune-Tumor Interactions
Study mechanisms of Killing

Enhanced T cell Kinetics
Monitor migration and infiltration

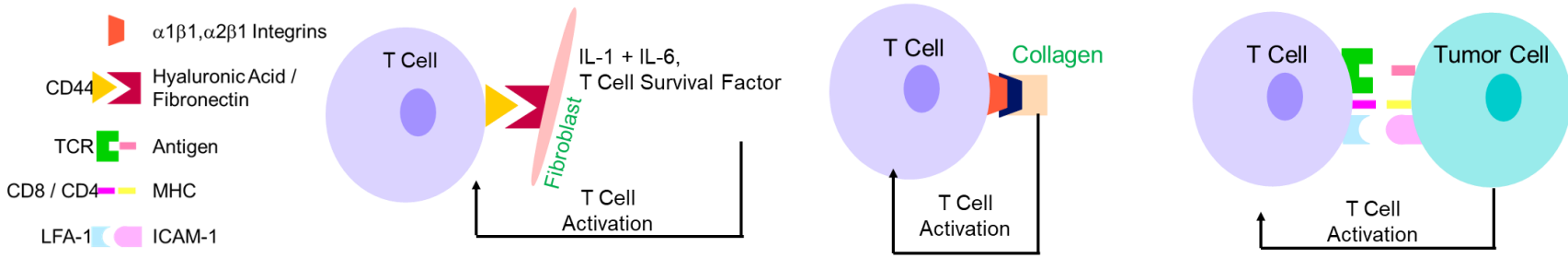
Immunosuppression
study mechanism of resistance

Tumor-Stroma
Fibroblast factors





Observed Lymphocyte-Tumor Interactions

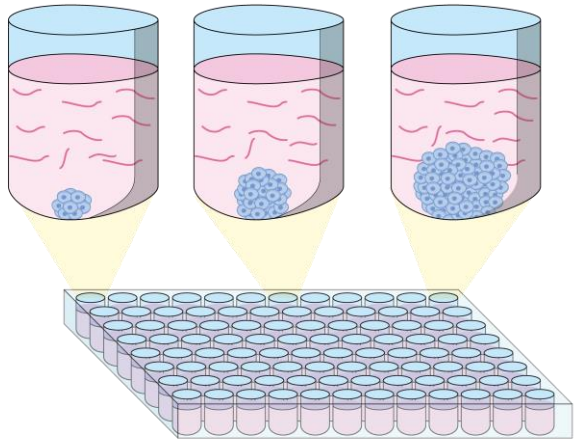


Comparison to Spheroids

IMMUNE 3D®

- Uniformity of spheroids
- Not all tumors create spheroids
- Multi-cell evaluations

Tumor Spheroids



- ✓ Layered – not spheroid
- ✓ Multi-cell evaluations
- ✓ Migration and Infiltration

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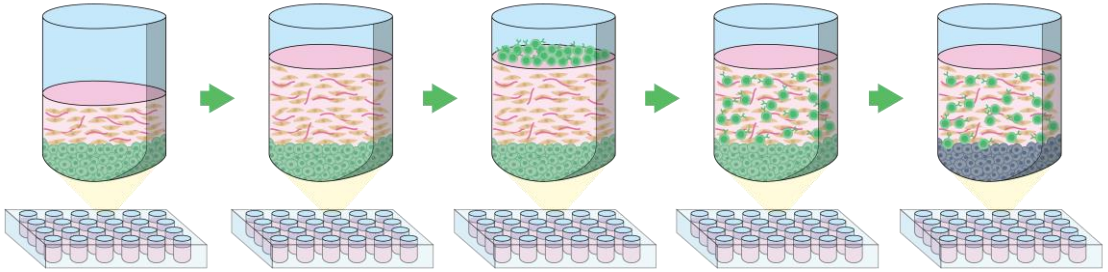

Comparison to Spheroids

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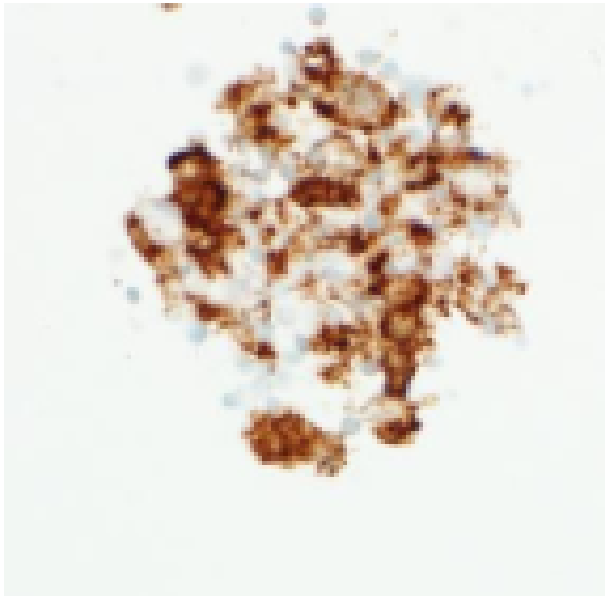
Enhanced Imaging to Monitor T cell Migration and Infiltration

Tumor Spheroids



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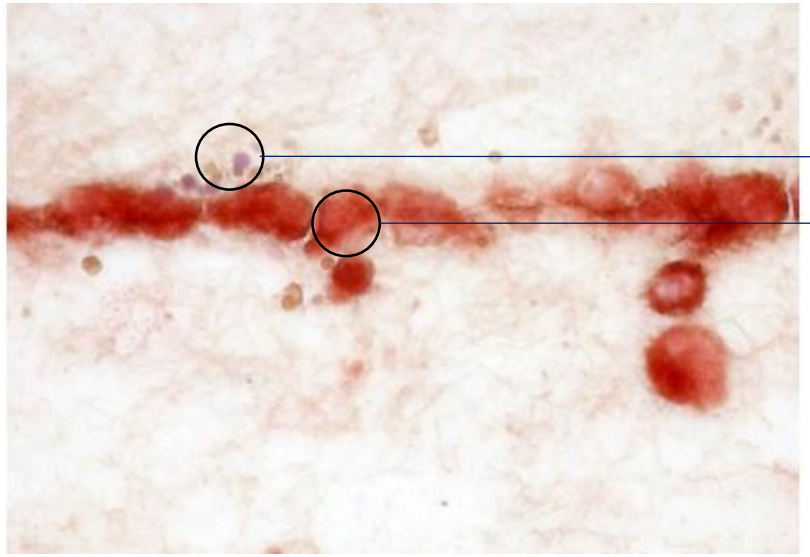

How does it work?

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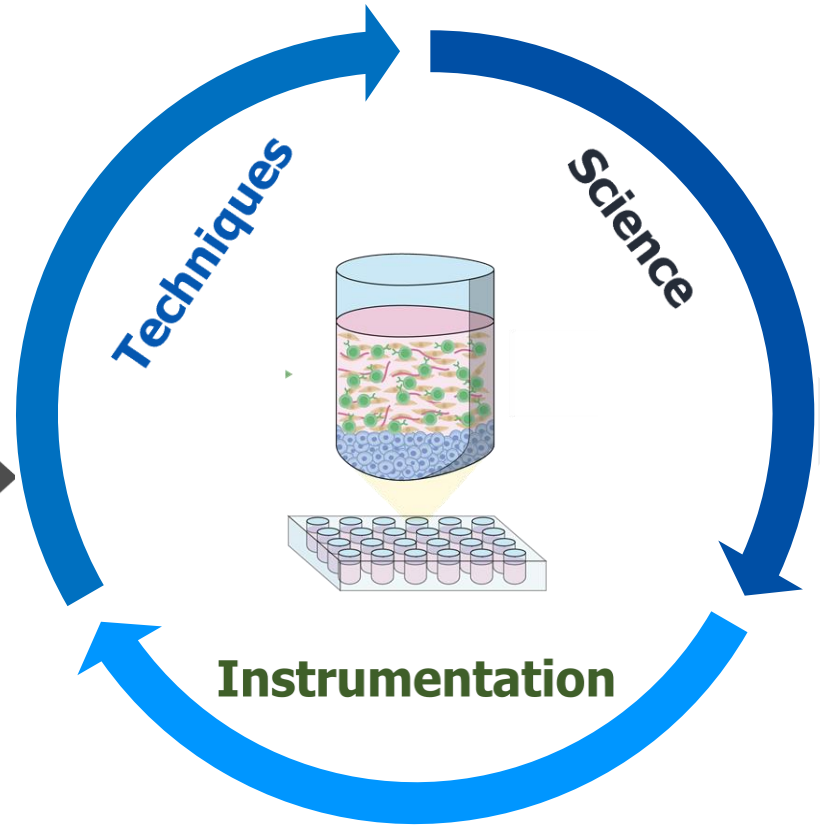
Simple Inputs and Valuable Outputs

Cell Platforms

- PDL1 sensitive
  HLA matched CTL and Tumor
- PD1 sensitive
  HLA matched CTL and Tumors
- Keytruda Resistant
  TIL and Tumor



Outcomes

- Mechanism of Action
- Function
- Efficacy
- Toxicity
- Dose

Applications

- Lead Selection
- Indication Expansion
- Combinations
- Repurposing
- Biomarkers

Advantages of Cell Platforms

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PBMC and Tumors

VS

- Non-specific readouts/responses
- Allogenic responses
- Single us experiments

Immunaccel

SPECIFICITY
Antigen-Specific T cell responses

CHARACTERIZED
Known profiles = Valuable "Inputs"

REPEAT USE
Across Assay and Models

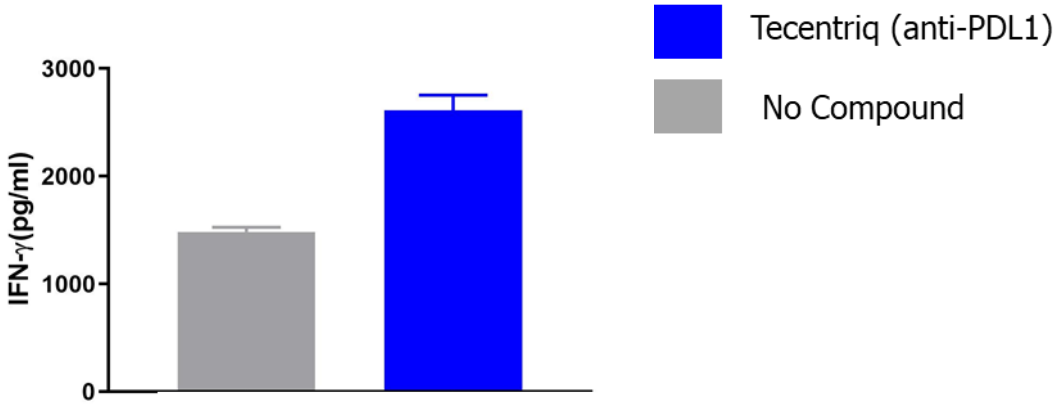
VALIDATION DATA
Compared to Approved Therapies

BROAD SPECTRUM
Readouts Applicable Across Tumors

PDL1 Sensitive Cell Platform (Colon)

IMMUNE 3D®

- HLA-matched CTL and Tumors generated from the same patient
- Extensive 2D and 3D studies conducted with these cell lines



CTL 001	Tumor 001
Cell Surface	**Cell Surface**
TIM-3, PD-1, CD25, CD80, HVEM, GITR, CD73, PD-L2, OX-40L, CD155, CD112, CD44, CD49a (α1 integrin), CD49b (α2 integrin), CD29 (β1 integrin), CD95 (FAS) CD54 (ICAM-1), CD11a (LFA-1a)	PD-L1, HVEM, OX-40L,B7-H3, CD155, CD112, CD44, CD49b(α2 integrin), CD29(β1 integrin), CD95 (FAS)
Chemokines	**Chemokines**
CXCR4,CCR7,CCR9, CXCL4,CXCL1-3	CXCL10,CX3CR1,CXCR2, CXCR3B,CCL21,CCL25, CXCL9

Cytotoxic T cell Lymphocytes (CTLs) are immune effector cell population that medicate specific immune responses against cancer.

PD1 Sensitive Cell Platforms

IMMUNE 3D®

Colon

CTL 002

Cell Surface

PD-1, TIGIT, OX-40, 4-1BB, CD80, CD44, CD95, CD29(β1 integrin), CD3, TCR α/β, CD95 (FAS), CD54 (ICAM-1), CD11a (LFA-1a)

Chemokines

CXCR4,CCR7,CCR9,CXCL4, CXCL1-3,CCR1-3,CCR5-7, CXCR1-5

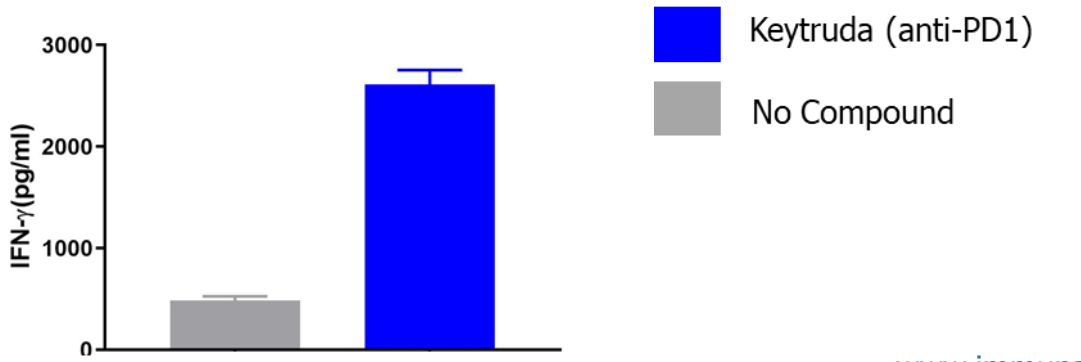
Tumor 002

Cell Surface

PD-L1, HVEM, OX-40L, CD155, CD112, CD44,CD49a (α1 integrin), CD49b (α2 integrin), CD29 (β1 integrin), CD95 (FAS)

Chemokines

CXCL10,CXCL17,CCL21, CCL25,CXCL9



Melanoma

CTL 003

Cell Surface

PD-1, TIGIT, OX-40, 4-1BB, CD80, CD44, CD95, CD29(β1 integrin), CD3, TCR α/β, CD95 (FAS), CD54 (ICAM-1), CD11a (LFA-1a)

Chemokines

CXCR4,CCR7,CCR9,CXCL4, CXCL1-3,CCR1-3,CCR5-7, CXCR1-5

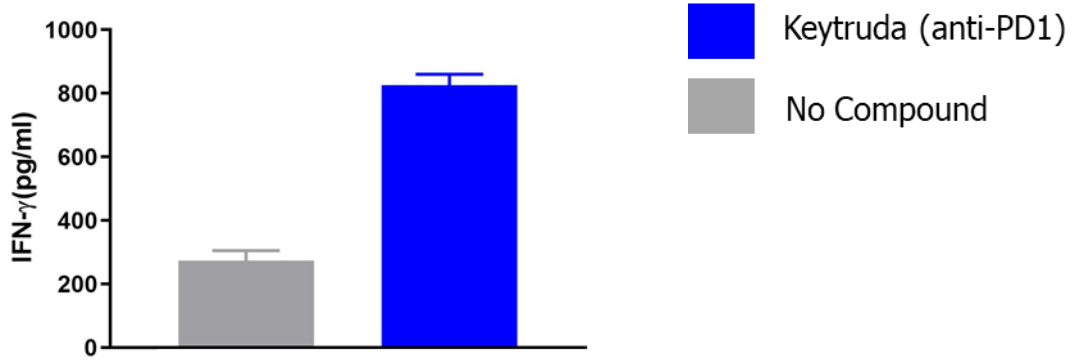
Tumor 003

Cell Surface

PD-L1, HVEM, OX-40L, CD155, CD112, CD44,CD49a (α1 integrin), CD49b (α2 integrin), CD29 (β1 integrin), CD95 (FAS)

Chemokines

CXCL10,CXCL17,CCL21, CCL25,CXCL9



PD1 Resistant TIL Platform (Melanoma)

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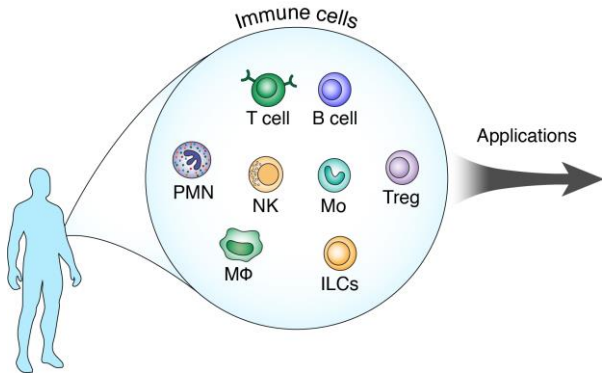
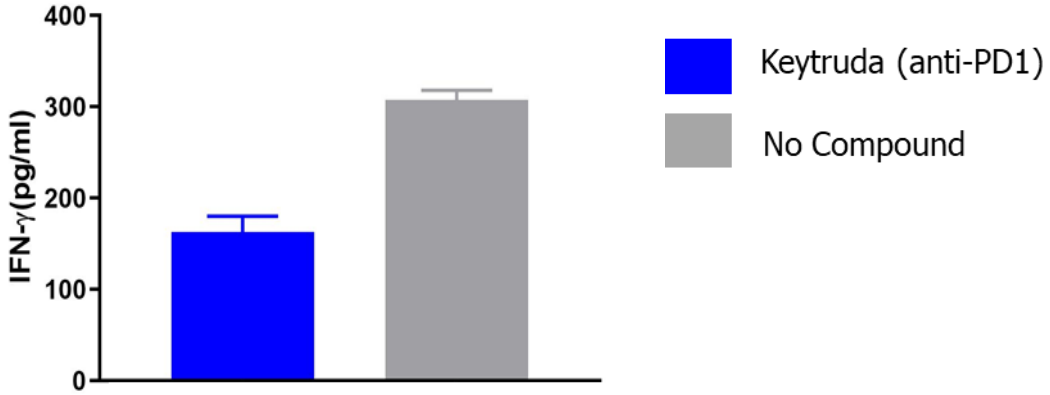
- TILs contain multiple immune cell populations
- Ideal for studying immunosuppression



Genomic Profile			
IMMUNO_START_DATE	11/23/2015		
IMMUNO_END_DATE	Currently ongoing		
IMMUNO_THERAPY_AGENT	Anti-PD1		
IMMUNO_BEST_RESPONSE	N/A		
MUTATIONAL INFORMATION			
	GENE	PROTEIN CHANGE	cDNA CHANGE
	TP53	p.P278L	c.833C>T
	NRAS	p.Q61K	c.181C>A
	BRAF	p.S467L	c.1400C>T

Combination of drug candidate with Keytruda faired better than Keytruda



TIL 004

Cell Surface

PD-1, TIM3, 41BB,CTLA4,CD45,CD27, TIGIT, GITR,OX40

Tumor 004

Cell Surface

41BBL,HVEM, CD112,CD155,CD44, CD49a (α1 integrin), CD49b (α2 integrin), CD29 (β1 integrin), CD95 (FAS)

**Contact us for sub-population data*

Our IMMUNE 3D Pipeline

IMMUNE 3D®

PD-1 Sensitive
- HLA-matched CTL 002/Tumor 002 (colon)
- HLA-matched CTL 003/Tumor 003 (mel.)

PDL1 Sensitive
- HLA-matched CTL 001/Tumor 001(colon)

Keytruda (anti-PD1) Resistance
- Using our TIL 004 and Tumor 004 we can study resistance mechanism and immune subpopulations such as Tregs and TAMs

Patient-derived Immune-Tumors
- We can use patient-derived cells from broad tumors such as lung, breast, ovarian

Skin Immunology*
- Evaluate the role of skin-γδ immune cells

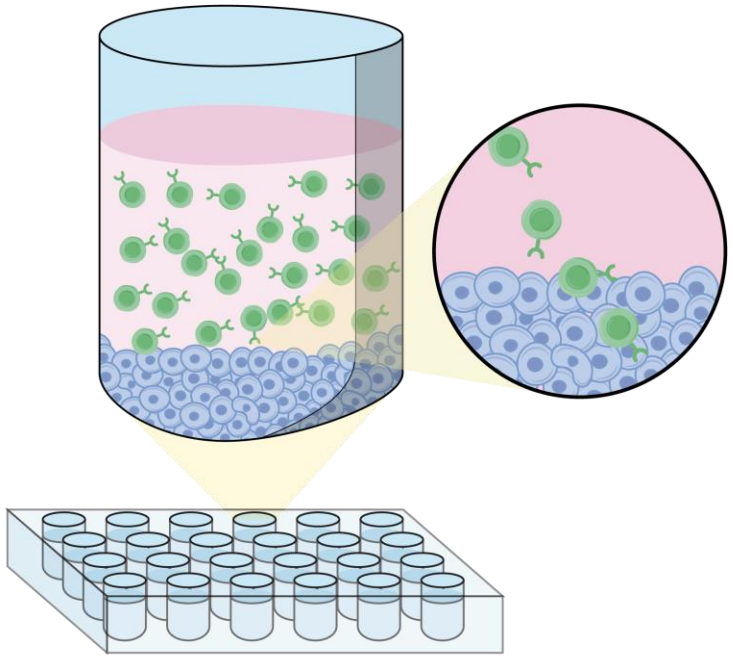
Microbiome*
- Evaluating interactions of gut microbes and immune cells in the TME

READOUTS
- ✓ Migration
- ✓ Infiltration
- ✓ Stromal factors
- ✓ Cytokines
- ✓ Antigens
- ✓ Metabolism
- ✓ Immune cell markers
- ✓ Chemokines
- ✓ Signaling

**In development*

Immune Infiltration

IMMUNE 3D®



The effects of CCR5 inhibition on regulatory T-cell recruitment to colorectal cancer

S T Ward*[,1], K K Li[2], E Hepburn[2], C J Weston[2], S M Curbishley[2], G M Reynolds[2], R K Hejmadi[3], R Bicknell[4], B Eksteen[5], T Ismail[3], A Rot[4] and D H Adams[2]

Monocytic CCR2+ Myeloid Derived Suppressor Cells Promote Immune Escape By Limiting Activated CD8 T Cell Infiltration Into The Tumor Microenvironment

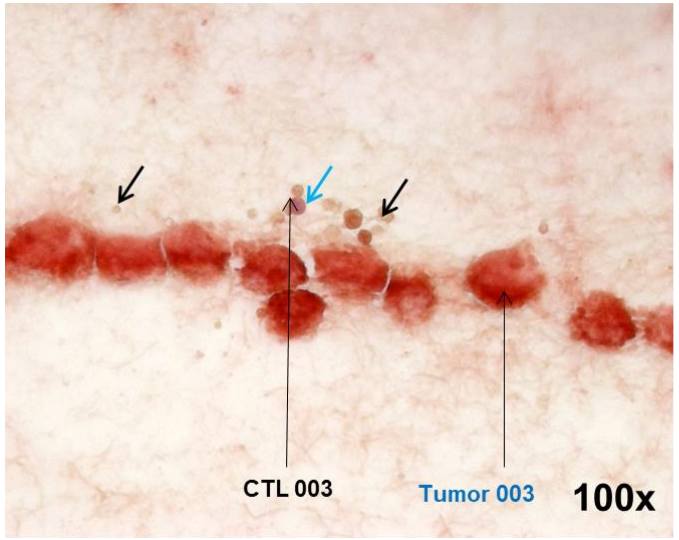
Alexander M. Lesokhin[1,2,*], Tobias M. Hohl[4,*], Shigehisa Kitano[1,8], Czrina Cortez[1], Daniel Hirschhorn-Cymerman[1], Francesca Avogadri[1], Gabrielle A. Rizzuto[5], John J. Lazarus[6], Eric G. Pamer[1,2,3], Alan N. Houghton[1,2,3], Taha Merghoub[1,†], and Jedd D. Wolchok[1,2,7,8,†]

Importance

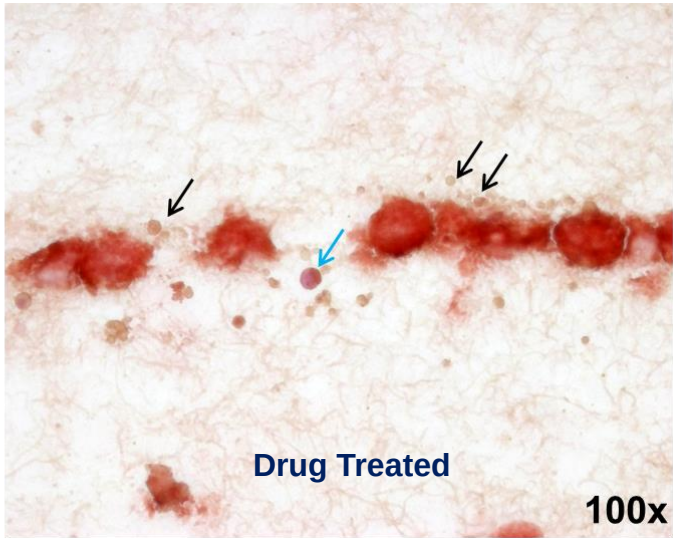
Clinical evidence has shown the presence or absence of T cell infiltrates in the TME to correlate with response to immune checkpoint blockade (anti-PD1/anti-PDL1) therapy

What we can do?

Screen and select drug candidates using our cell platforms, your clinical trial samples and patient-derived samples based on T cell or immune infiltrates

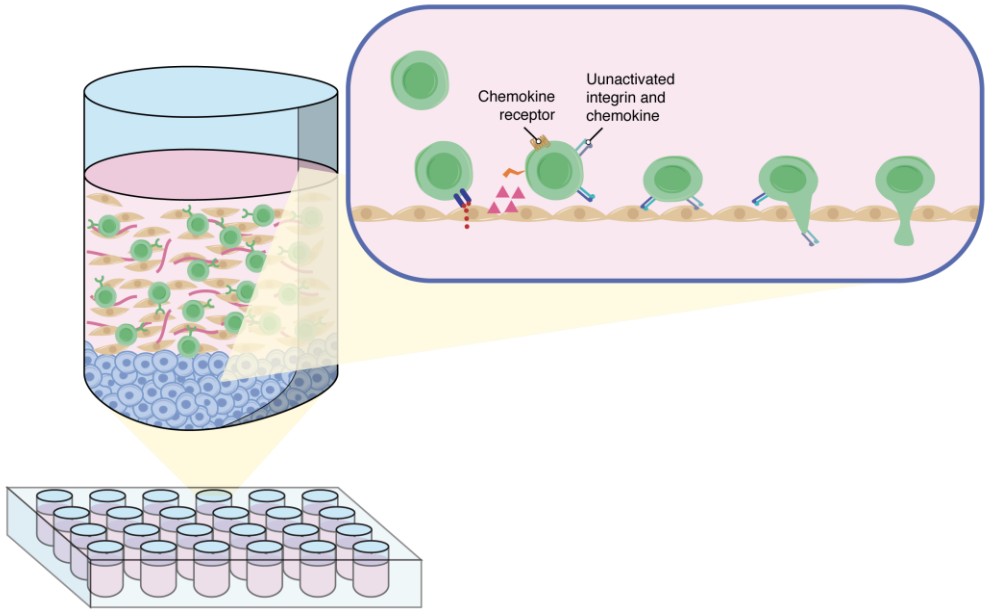


CTL003 and Tumor 003 infiltrates



CTL003 and Tumor 003 infiltrates with anti-PD1 drug treatment

Immune Migration



Improving homing in T cell therapy

Debora Vignali[a], Marinos Kallikourdis[a,b,*]

[a] Adaptive Immunity Laboratory, Humanitas Clinical and Research Center, Via Manzoni 56, Rozzano (Milano), Italy
[b] Humanitas University, Via Manzoni 113, Rozzano (Milano), Italy

Regulation of Chemokine Expression in the Tumor Microenvironment

Anton V Gorbachev, Robert L Fairchild

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Importance

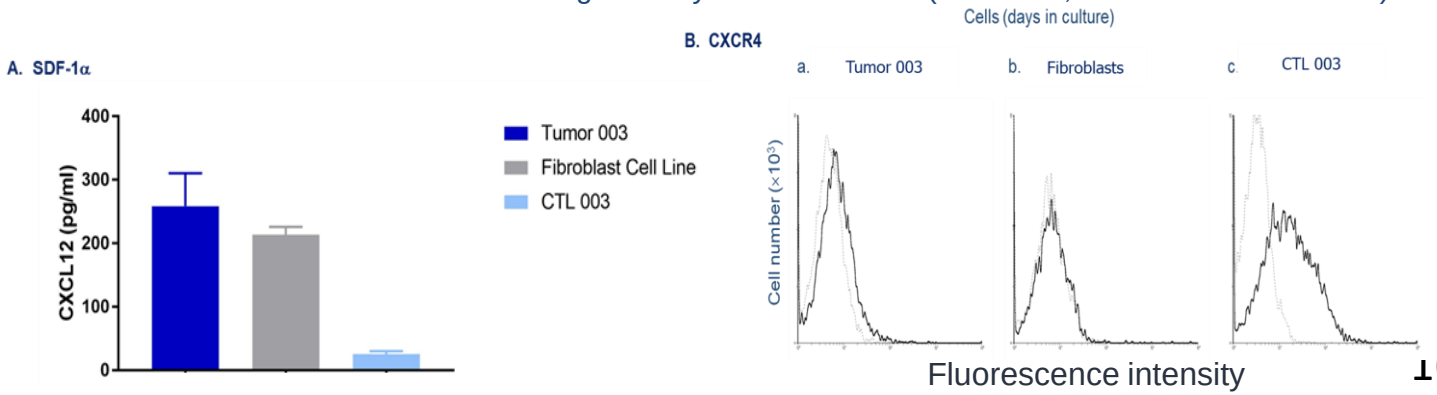
T cell migration is essential for T cell responses and antigen responses. Chemokines play a key role in migration

What we can do?

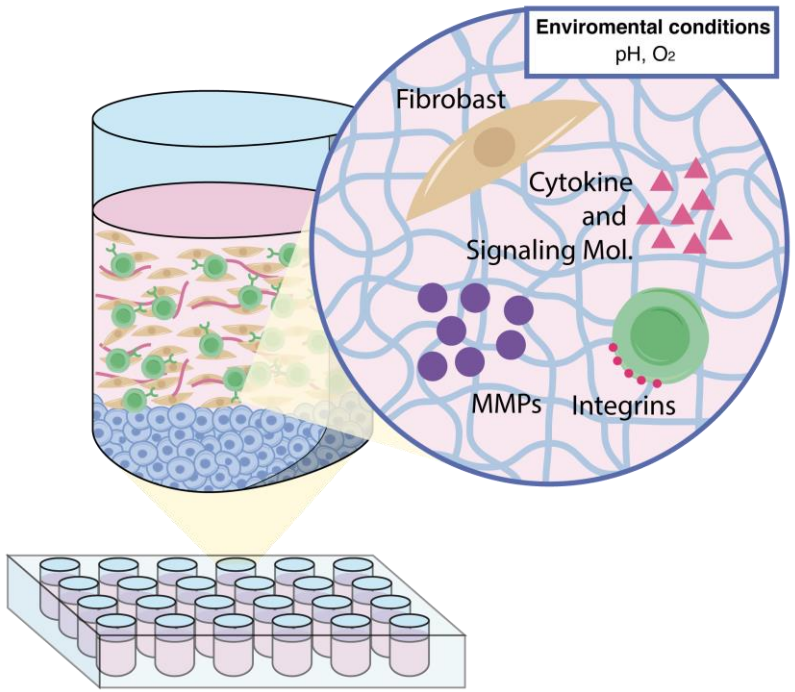
Screen and select drug candidates using our cell platforms and observe chemokine changes and measure apoptosis of tumors

Treatment	Total No. of tumor cells Mean ± SD/field (30 fields)	Percentage of apoptotic tumor cells Mean ± SD/field (30 fields)
Tumor 003	17.2 ± 4.5	6.5 ± 3.4
Tumor 003 + CTL003	11.5 ± 3.5	19.5 ± 8.3[a, b]
Tumor 003 + CTL003 + SDF-1α	15.6 ± 2.5	5.6 ± 4.1 [a]
Tumor 003 + CTL003 + AMD3100	18.0 ± 3.9	5.6 ± 4.1[b]
Tumor 003 + CTL003 + control Ig	11.4 ± 2.8	17.5 ± 5.2[c, d]
Tumor 003 + CTL003 + α-CXCR4 Ab	13.5 ± 2.6	4.8 ± 4.3[c]
Tumor 003 + CTL003 + a-SDF-1α Ab	12.7 ± 2.8	4.9 ± 4.6[d]

a, b, c and d Values with the same letter differ significantly from each other ($P < 0.001$, Student's 2-sided t-test).



Immune-Tumor Stroma



Tumor-associated stromal cells as key contributors to the tumor microenvironment

Karen M. Bussard[1,2], Lysette Mutkus[3], Kristina Stumpf[3], Candelaria Gomez-Manzano[4] and Frank C. Marini[1,3*]

Can targeting stroma pave the way to enhanced antitumor immunity and immunotherapy of solid tumors?

Ellen Puré and **Albert Lo**
University of Pennsylvania, Philadelphia, PA, USA

IMMUNE 3D®

Importance

Stromal cells may critically affect T cell homing and migration. Targeting stromal cells components may improved T cell homing and migration

What we can do?

Screen and select drug candidates using our cell platforms and characterize stromal factors that may play a role in facilitating T cell or immune cell migration

IMMUNE 3D captures CD, adhesion, integrins, apoptosis and checkpoint molecules

Parameter Investigated	Tumor 003		Tumor 001	
	2D	IMMUNE 3D ™	2D	IMMUNE 3D ™
CD4	+++	+++	+++	+++
CD8	++	++	+	+
CD40L	-	+	-	-
α1 integrin	-	-	-	-
α2 integrin	-	-	-	-
β1 integrin	+	+++	+++	+++
FAS	++	+++	+++	+++
FASL	-	++	++	-
ICAM-1	+	++	+++	+++
LFA-1	+	+++	+++	+++

Tumor 003 and Tumor 001 stromal factors under IMMUNE 3D conditions

Immune-Metabolism

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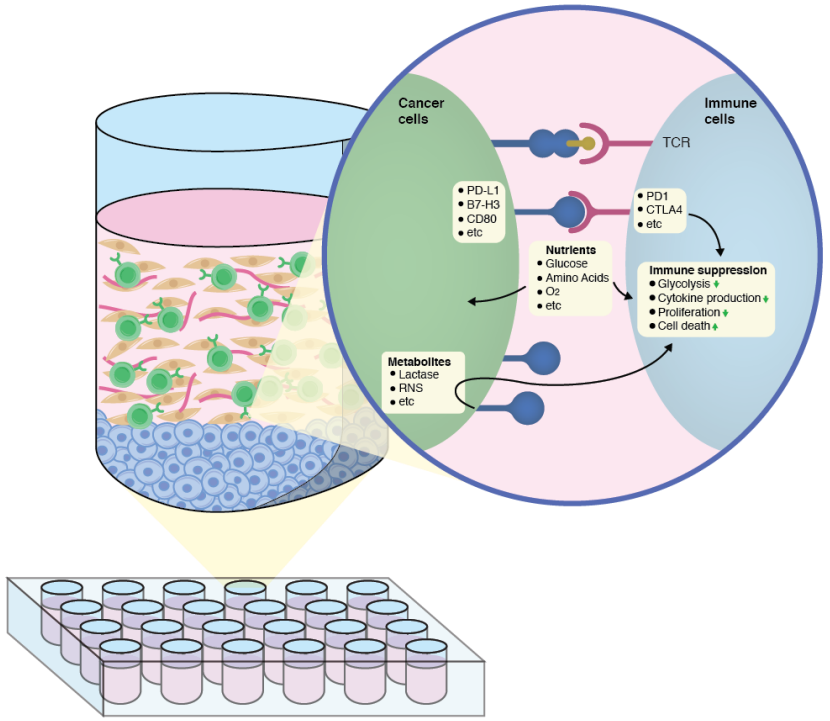

Emerging concepts of T cell metabolism as a target of immunotherapy

Chih-Hao Chang[1] & Erika L Pearce[2]

Interplay between Immune Checkpoint Proteins and Cellular Metabolism

Sangbin Lim[1], Joshua B. Phillips[1], Luciana Madeira da Silva[1], Ming Zhou[2], Oystein Fodstad[3], Laurie B. Owen[1], and Ming Tan[1]

Importance

Understanding metabolic programming in the tumor microenvironment may serve as important factors in evaluating immune responses

What we can do?

Design a hypoxic environment using our cell platforms and measure metabolic signatures and factors such hypoxic-inducible factor 1 alpha (HIF-1$^{\alpha}$)

Immuno-metabolism data available on request

Antigen Presentation

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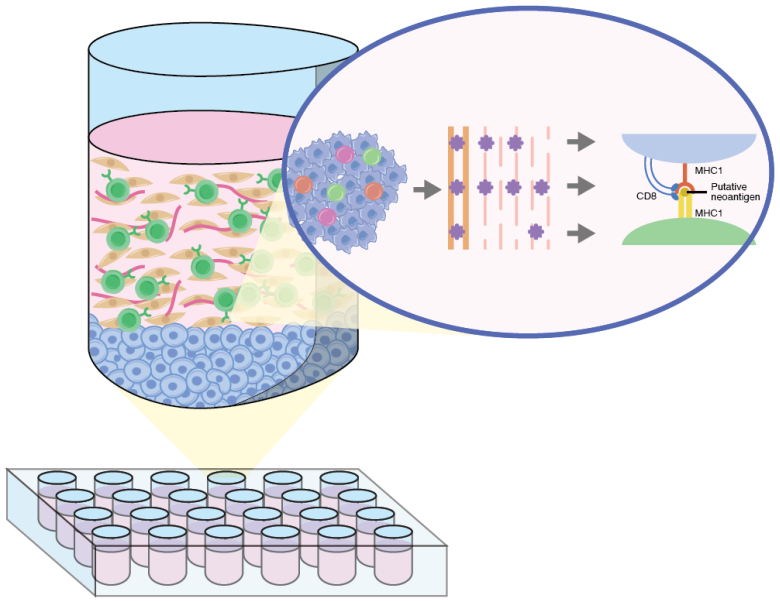


Neoantigens in cancer immunotherapy

Ton N. Schumacher[1]* and Robert D. Schreiber[2]*

Loss of CTL Function among High-Avidity Tumor-Specific CD8 + T Cells following Tumor Infiltration

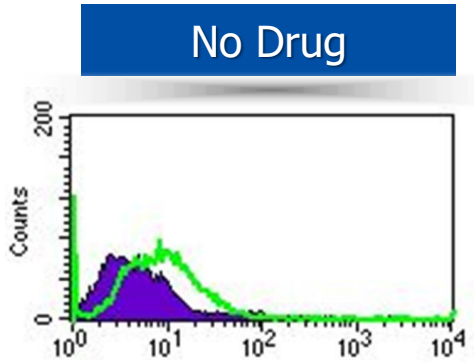
Claire N. Janicki, S. Rhiannon Jenkinson, Neil A. Williams, et al.

Importance

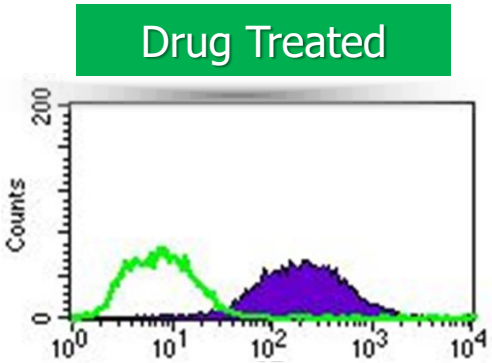
T cell infiltration and migration may depend on the presence of neoantigens and tumor heterogeneity

What we can do?

- Introduce point mutations in our tumor cell platforms to observe CTL infiltration and migration patterns and screen for T cell-tumor interactions
- Screen and select drug candidates based on enhancing antigen presentation

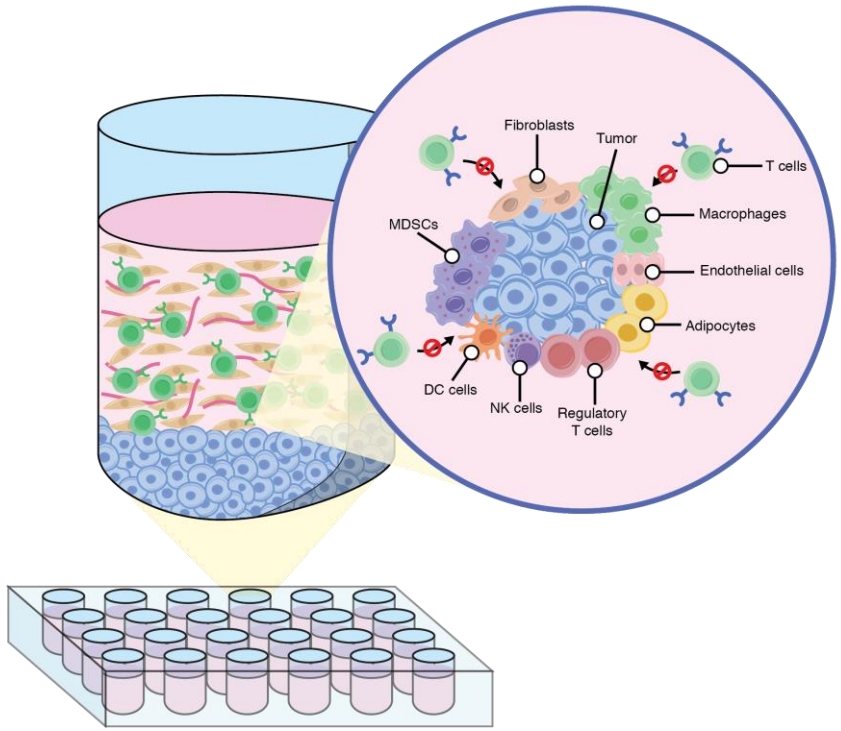


Low antigen presentation on Tumor 002



Checkpoint treatment enhances antigen presentation on Tumor 002

Immunosuppression



Facilitating T Cell Infiltration in Tumor Microenvironment Overcomes Resistance to PD-L1 Blockade

Haidong Tang,[1,2] Yang Wang,[1,2] Lukasz K. Chlewicki,[1] Yuan Zhang,[1] Jingya Guo,[3] Wei Liang,[3] Jieyi Wang,[4] Xiaoxiao Wang,[5] and Yang-Xin Fu[2,3,*]

Suppression of T Cell Responses in the Tumor Microenvironment

Alan B Frey

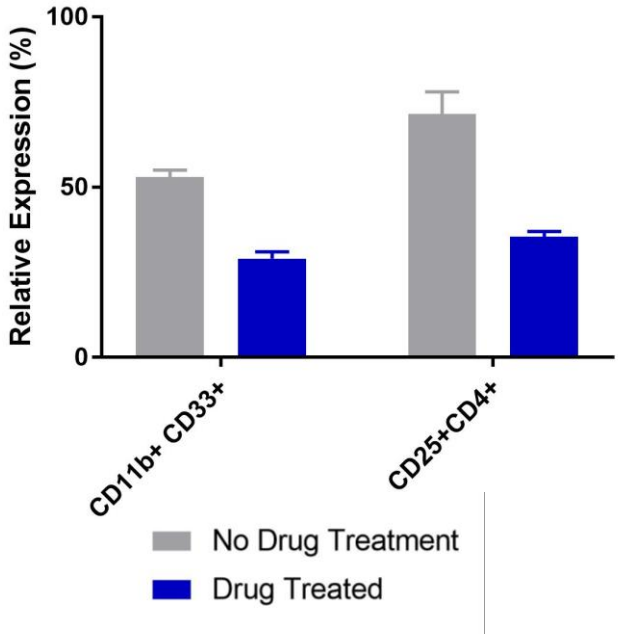
IMMUNE 3D®

Importance

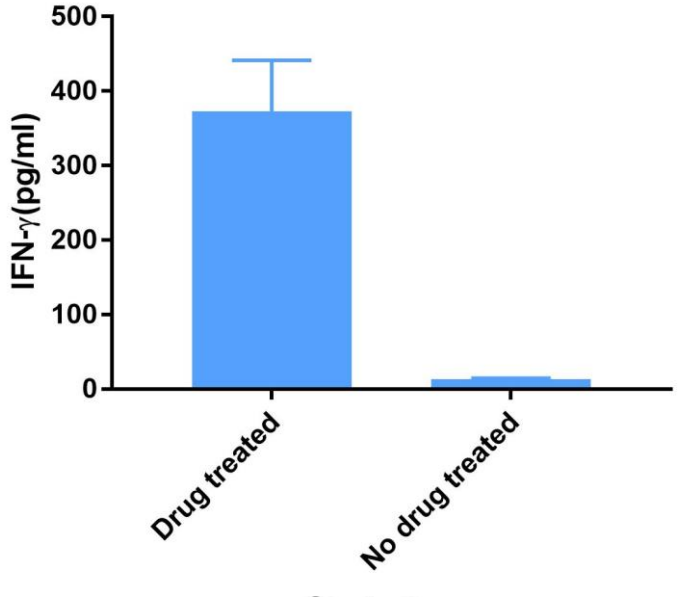
Certain immune cell populations such as Tregs and MDSCs inhibit T cell migration and disrupt antigen presentation

What we can do?

Screen and select drug candidates using our Keytruda Resistant TIL and tumor that contain immunosuppressive sub-populations and measure cell surface and cytokine changes in response to drug treatment



Measured changes in Myeloid and Treg markers in response to checkpoint blockade therapy



Cytokine responses in response to checkpoint therapy

Inhibitory Receptors

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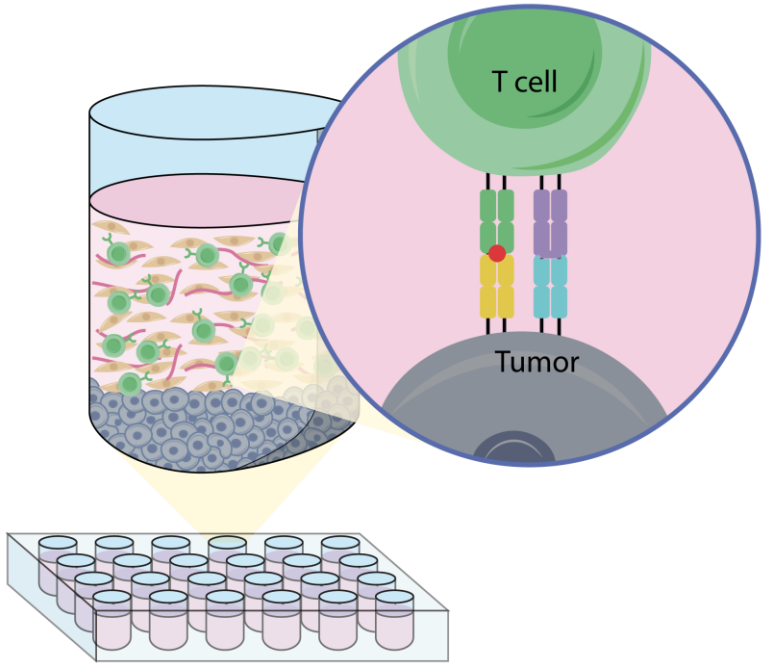


The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints

Nicolas J. Llosa[1], Michael Cruise[2,7], Ada Tam[3], Elizabeth C. Wick[4], Elizabeth M. Hechenbleikner[4], Janis M. Taube[3], Lee Blosser[4], Hongni Fan[1], Hao Wang[5], Brandon Luber[5], Ming Zhang[6], Nickolas Papadopoulos[6], Kenneth W. Kinzler[6], Bert Vogelstein[6,7], Cynthia L. Sears[1,8], Robert A. Anders[2], Drew M. Pardoll[1,2,7,8,*], and Franck Housseau[1,*]

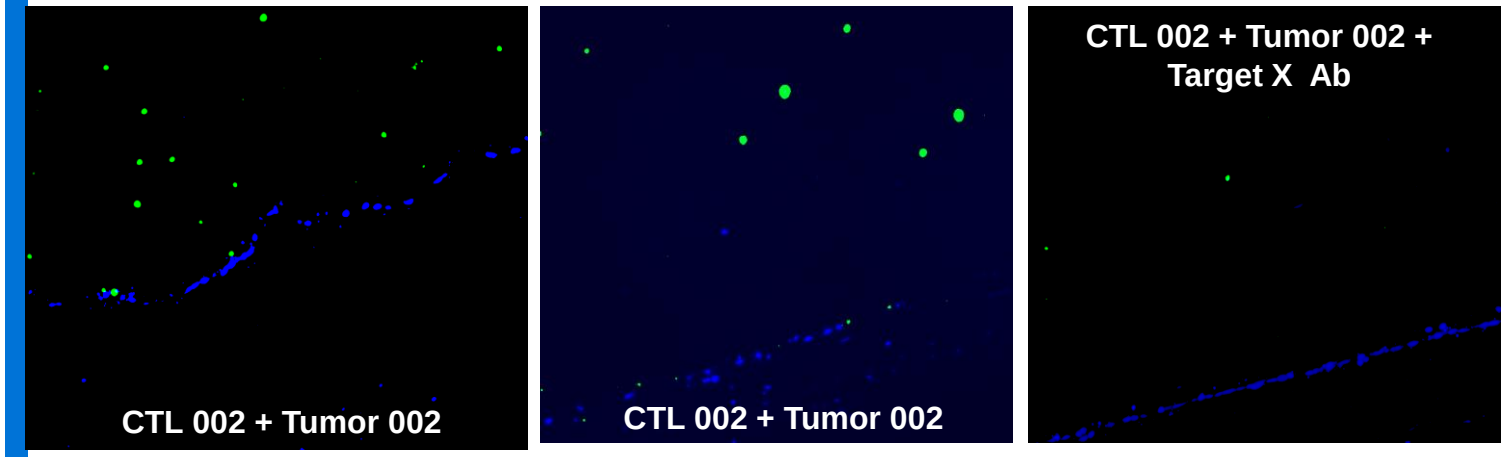
Importance

Inhibitory signaling pathways present on T cells and tumors impede effector T cell functions and tumor lysis

What we can do?

Screen and select drug candidates using our cell platforms based on receptor/ligand expression on CTL and blocking studies to measure on tumor lysis

We screened for an undisclosed receptor on CTL 002 (colon) and conducted blocking studies targeting receptor on CTL 002



Biomarkers

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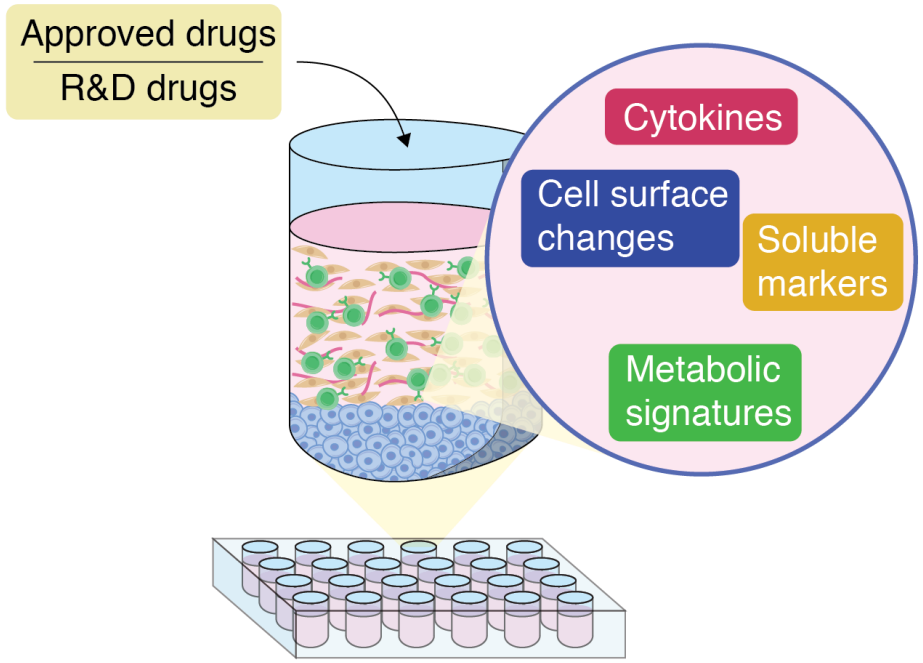


Immunotype and Immunohistologic Characteristics of Tumor Infiltrating Immune Cells are Associated with Clinical Outcome in Metastatic Melanoma

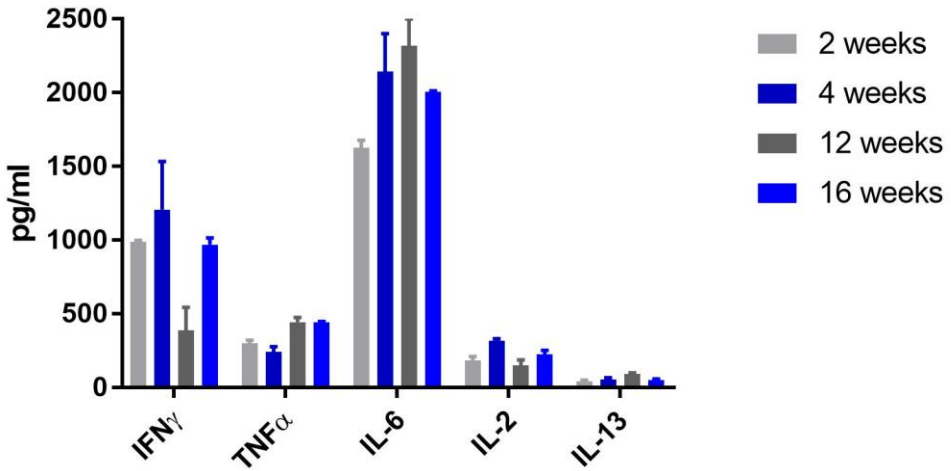
Gulsun Erdag[1,2,*], Jochen T. Schaefer[1,2,**], Mark E. Smolkin[3], Donna H. Deacon[2], Sofia M. Shea[2], Lynn T. Dengel[2], James W. Patterson[1], and Craig L. Slingluff Jr.[2]

Importance

Biomarkers can aide in determining patient response to a drug candidate

What we can do?

Screen drug candidates using our cell platforms and monitor changes in cytokine levels or other markers across various time points



IL-6 in response to a checkpoint blockade drug candidate using CTL 002 and Tumor 002 (colon)

Team

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Applications Across the Translational Spectrum

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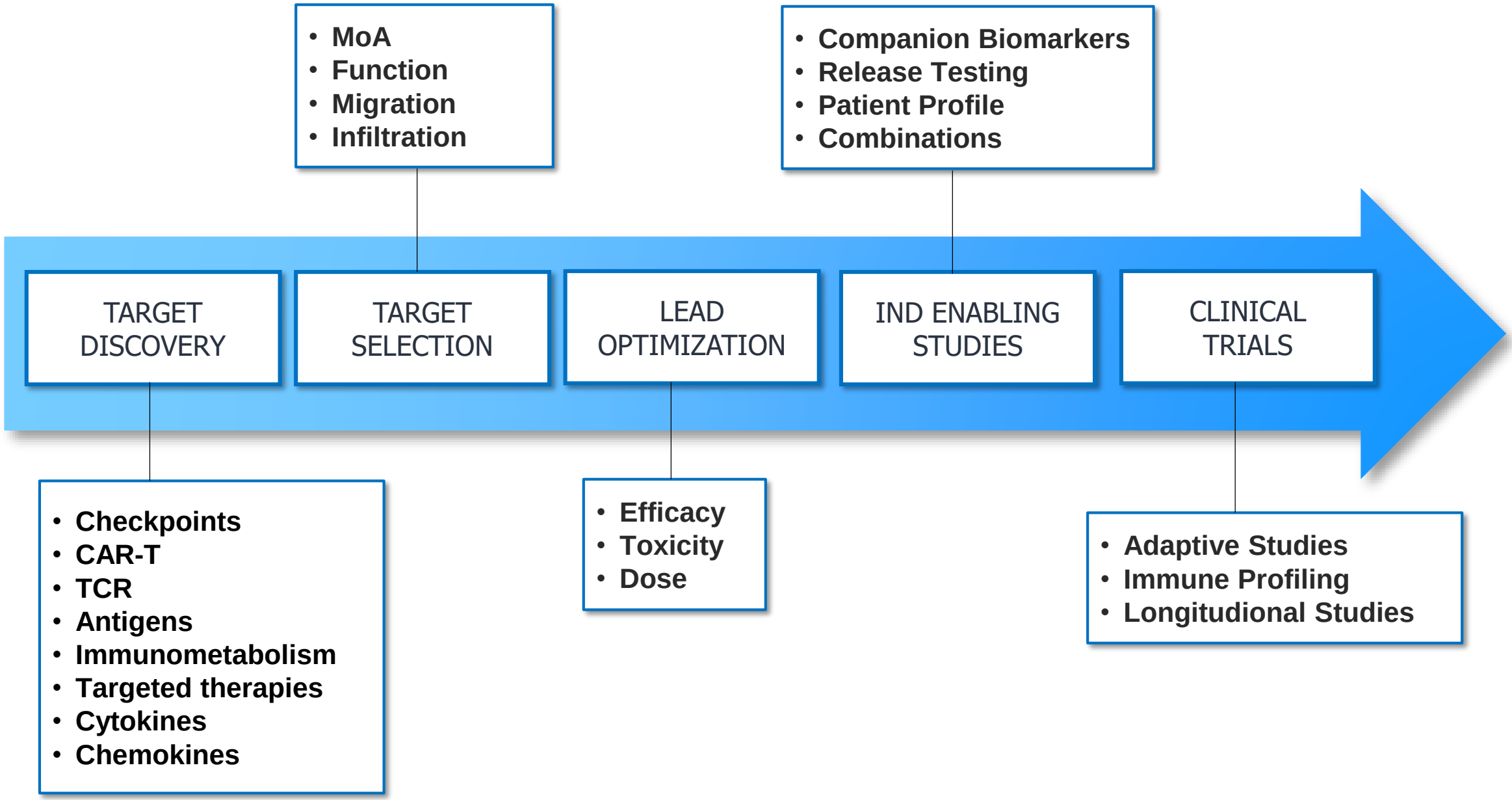

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