

Immune Monitoring for Translational Clinical Trials

Julie M. Curtsinger Ph.D.
Coordinator
Translational Therapy Core Facility
Masonic Cancer Center
University of Minnesota

Translational Therapy Core Facility

- Receive, process, test, and store research specimens from patients enrolled in clinical trials
- Currently provide support for >30 clinical trials
- Services are distinct from those provided by a clinical lab
- Wide range of services provided, including full range of immune monitoring assays for clinical trial

Basics of Immune Monitoring

- Addresses research or correlative goals of immunotherapy trials
- May be as important as clinical goals
- Requires significant up front planning and preparation
- Should be ready to adapt to unexpected events once trial is open

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Planning for Immune Monitoring

- What are the research questions?
- What assays will be used to answer the research questions?
- What type and size of tissue samples are needed for the planned assays?
- What will the interval of sample collection be?
- Will assays use fresh or cryopreserved samples?
- What are testing priorities if sample size is limited?
- Who will do the work?
- How and when will results be reported?

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Planning for Immune Monitoring

- **What are the research questions?**
 - Defined by Principal Investigators of the clinical trial
 - Written in the clinical trial protocol

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Planning for Immune Monitoring

- **What assays will be used to answer the research questions?**
 - Can assays used in preclinical research be used for clinical research?
 - Can assays be modified to make them work for clinical research testing?

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Planning for Immune Monitoring

- **What type and size of tissue samples are needed for the planned assays?**
 - If tissue is blood, will it be collected with anticoagulant? If so, which one?
 - Will the planned volume of blood provide the necessary amount of testing material- serum, cells, etc.?

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Planning for Immune Monitoring

- **What will the interval of sample collection be?**
 - Frequent samples after initial therapy?
 - Serial samples over an extended period?

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Planning for Immune Monitoring

- **Will assays use fresh or cryopreserved samples?**
 - If fresh cells will be used, must be prepared to test whenever samples show up in the lab
 - If cryopreserved cells will be used, must validate assays by direct comparison of results with same cells fresh and thawed

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Planning for Immune Monitoring

- **What are testing priorities if sample size is limited?**
 - Prioritize testing in advance

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Planning for Immune Monitoring

- **Who will do the work?**
 - Core facility
 - Research lab
 - Combination of the two

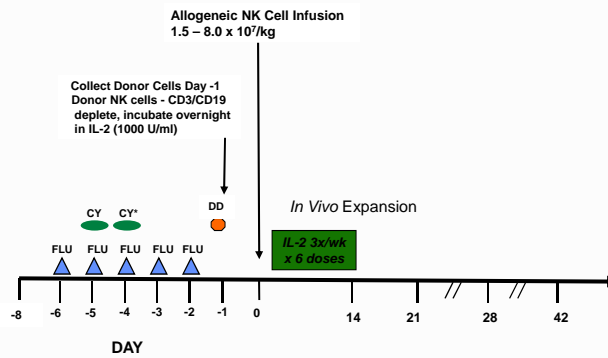
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Planning for Immune Monitoring

- **How and when will results be reported?**
 - Maintain records to show compliance with research sample collection schedule
 - Plan in advance how to provide results to PI

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Immune Monitoring: NK trials



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Immune Monitoring: NK trials

- **Research questions:**

- **Product:**

- Do the activated NK cells have cytotoxic effector function?

- **Patient:**

- What are levels of cytokines IL-7 and IL-15 in serum?
 - Do the infused NK cells expand in vivo?
 - What is phenotype of NK cells?
 - Do NK cells in blood have cytotoxic effector function?

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Immune Monitoring: NK trials

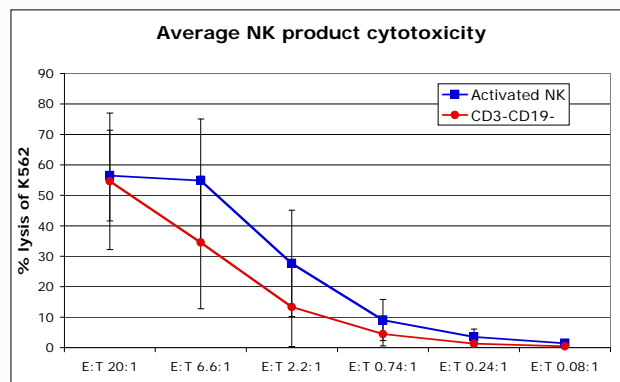
Sample grid		Flow cytometry phenotype	Cytotoxicity assay	Freeze cells	Freeze serum
Product	CD3-CD19-	X	X	X	
	Activated NK	X	X	X	
Patient	Pre-chemo	X	X	X	X
	Pre-NK infusion	X	X	X	X
	Day 7	X	X	X	X
	Day 14	X	X	X	X
	Day 28	X	X	X	X
	2 months	X	X	X	X

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Immune Monitoring: NK trials

- **Product:**

- Do the activated NK cells have increased cytotoxic effector function?

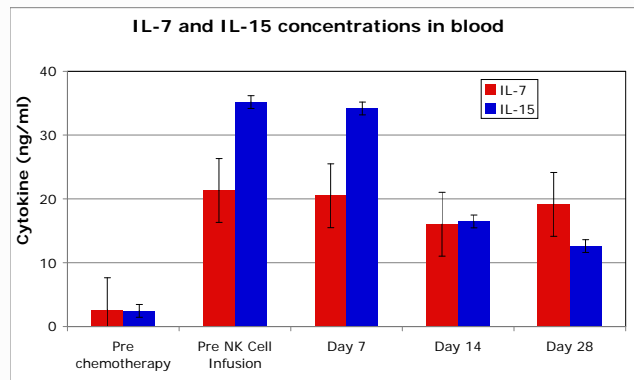


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Immune Monitoring: NK trials

- **Patient:**

- What are concentrations of IL-7 and IL-15 in serum?

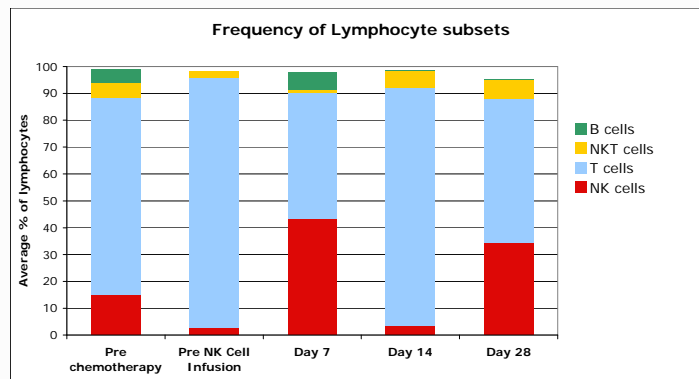


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Immune Monitoring: NK trials

- **Patient:**

- Do infused NK cells expand in vivo?

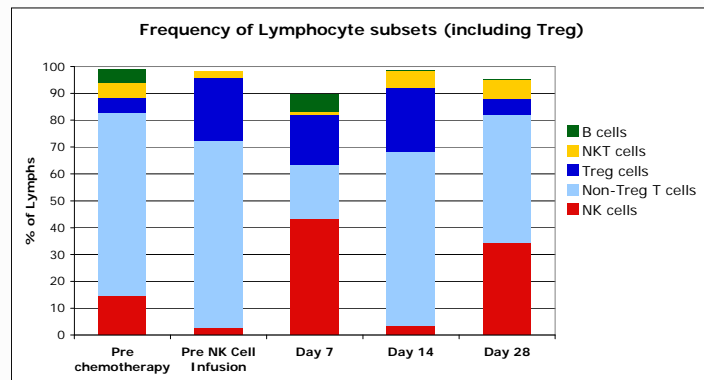


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Immune Monitoring: NK trials

- **Patient:**

- Do infused NK cells expand in vivo?



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Immune Monitoring: NK trials

- **Research questions- revised:**

- **Product:**

- Do the activated NK cells have effector function?

- **Patient:**

- Do the infused NK cells expand in vivo?
- Do regulatory T cells (Treg) expand in vivo?
- What is proliferation status of lymphocyte subsets?
- What receptors are expressed on NK cells?

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Immune Monitoring: NK trials

Sample grid		9-color Foxp3-Ki67 phenotype	9-color NK phenotype	Cytotoxicity assay	Freeze cells	Freeze serum
Product	Apheresis product	X			X	
	Activated NK	X	X	X	X	
Patient	Pre-chemo	X			X	X
	Pre-NK infusion	X			X	X
	Day 7	X	X		X	X
	Days 14 & 21	X	X	X	X	X
	Days 28, 42, 60, 100	X	X		X	X
	Months 6, 9, & 12				X	X

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Immune Monitoring: NK trials

- Do the infused NK cells expand in vivo?
- Do regulatory T cells (Treg) expand in vivo?
- What is proliferation status of lymphocyte subsets?

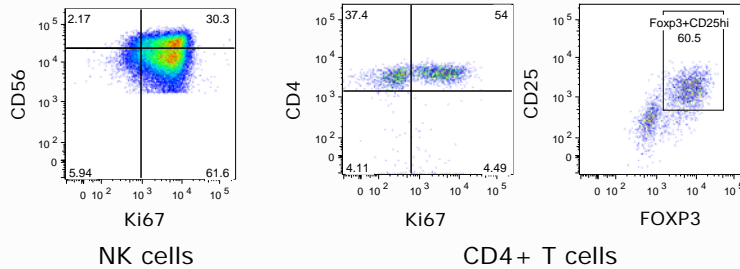
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Immune Monitoring: NK trials

9 color phenotype panel

AF700	ECD	PE-Cy7	PE	APC	AF488	Pac Blue	PerCP-Cy5.5	V500
CD19	CD3	CD56	Ki67	CD25	Foxp3	CD8	CD4	CD45

Patient sample: Day 7 post-NK cell infusion



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Immune Monitoring: NK trials

9 color panel to assess dual NK function

Alexa700	PE-TexasRed	PE-Cy7	PE	APC	FITC	Pacific Blue	PerCP-Cy5.5	V500
NKB1	CD3	CD56	NKG2A	CD158a	CD158b	IFNg	CD107a	CD45

Assay (can use cryopreserved cells):

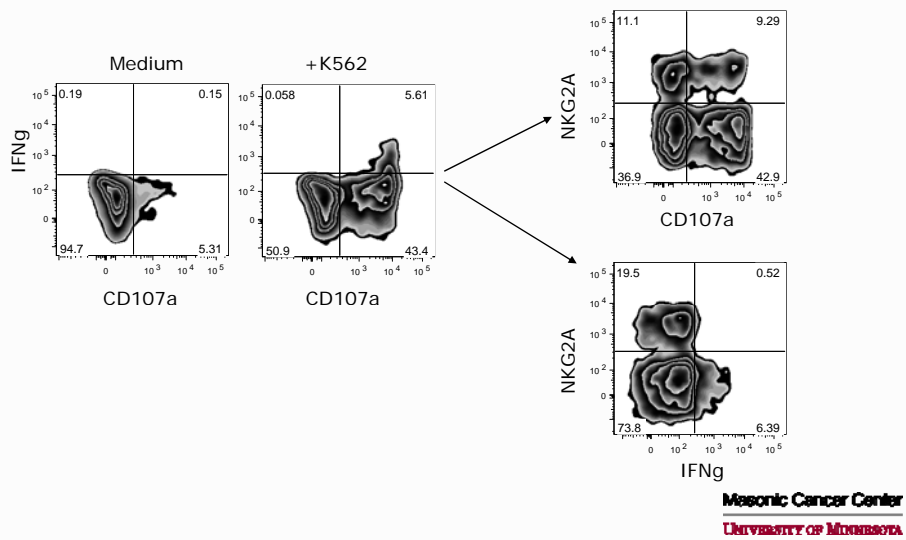
- Culture PBMC in Medium alone or with K562 target cells for 5 hours
- Stain using 9 color panel
- Analyze with flow cytometer

Result:

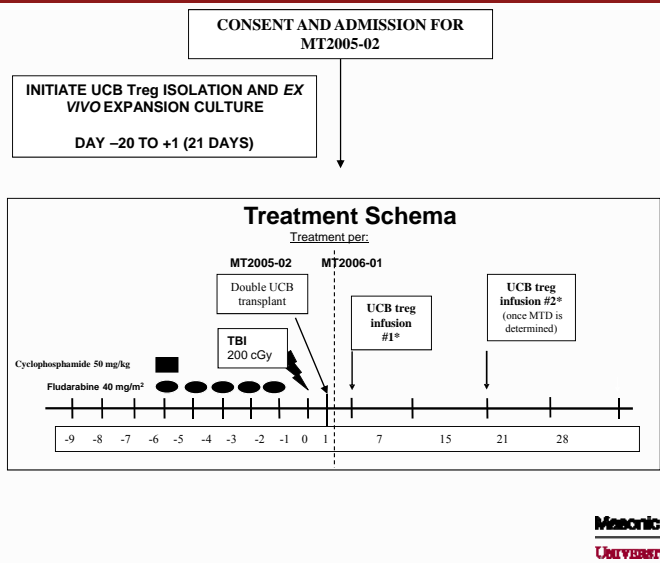
- NK cells that can lyse K562 cells will be detected by the presence of CD107a on surface following fusion of lytic granules with cell membrane
- NK cells that can produce IFNg when they encounter K562 cells will be detected by intracellular staining with anti-IFNg antibody
- Allows functional assessment of NK subpopulations

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Immune Monitoring: NK trials



Immune Monitoring: Treg trial



Immune Monitoring: Treg trial

- **Research questions:**

- What are the frequency and phenotype of Treg cells at various times post-transplant?
- Do the infused Treg cells expand in vivo?
- What is the suppressive activity of patient CD4+ cells after Treg infusion?
- What are levels of cytokines IL-7 and IL-15 in serum?

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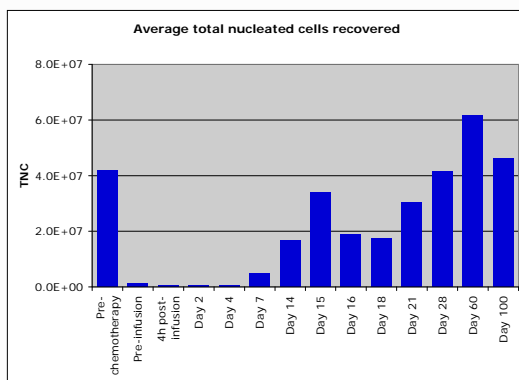
Immune Monitoring: Treg trial

Sample	Whole blood phenotype	Treg phenotype	Suppression assay	Freeze cells	Freeze serum
Pre-chemo	X	X		X	X
Pre-infusion	X	X		X	X
4h post infusion	X	X		X	X
2 days post	X	X		X	X
4 days post	X	X		X	X
7 days post	X	X	X	X	X
14 days post	X	X	X	X	X
28 days post	X	X	X	X	X
60 days post	X	X	X	X	X
100 days post	X	X	X	X	X

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Immune Monitoring: Treg trial

Low cell recoveries in the first two weeks post-transplant limited testing options



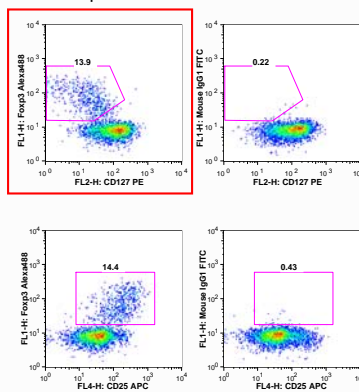
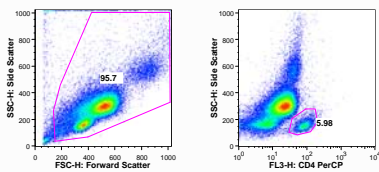
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Immune Monitoring: Treg trial

- What are the frequency and phenotype of total Treg cells post-infusion?

Gating to define Treg cells:

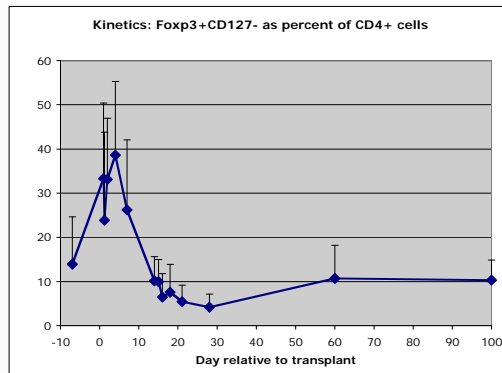
Treg cells are defined as CD4+Foxp3+CD127-



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Immune Monitoring: Treg trial

- What are the frequency and phenotype of total Treg cells post-infusion?



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Immune Monitoring: Treg trial

- Do the infused Treg cells expand in vivo?

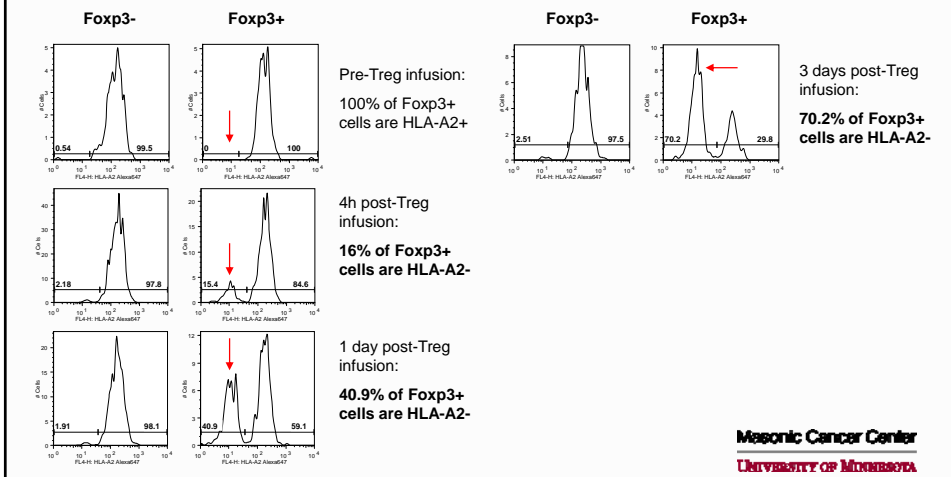
	Patient #9
Patient	A2 B15 A31 B51
Treg cord	A11 B51 A31 B15
Transplant #1	A2 B15 A31 B35
Transplant #2	A2 B35 A31 B51

Patient #9:
Infused Treg cells are
HLA-A2 negative

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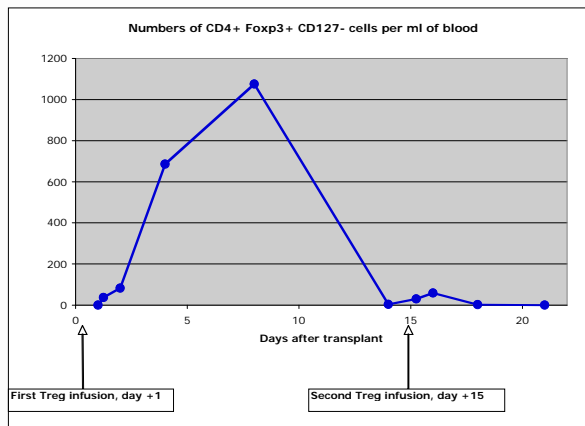
Immune Monitoring: Treg trial

- Do the infused Treg cells expand in vivo?



Immune Monitoring: Treg trial

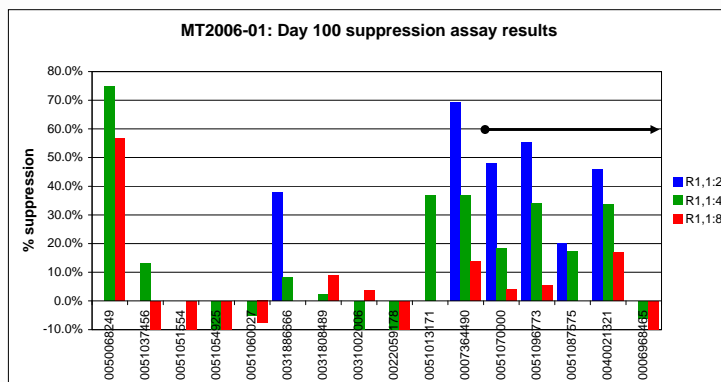
- Do the infused Treg cells expand in vivo?



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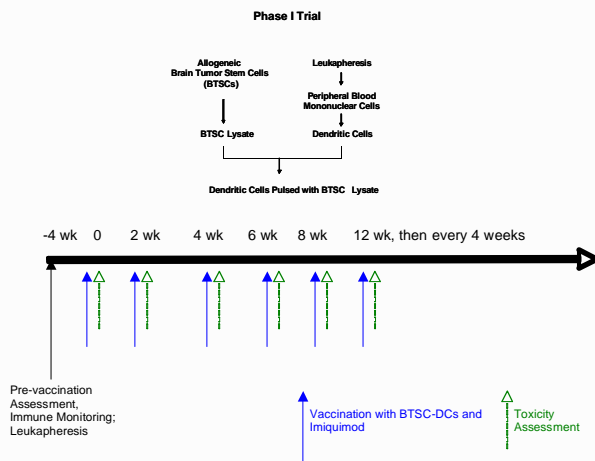
Immune Monitoring: Treg trial

- Do patient CD4+ cells suppress proliferation?



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Immune Monitoring: DC vaccine



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Immune Monitoring: DC vaccine

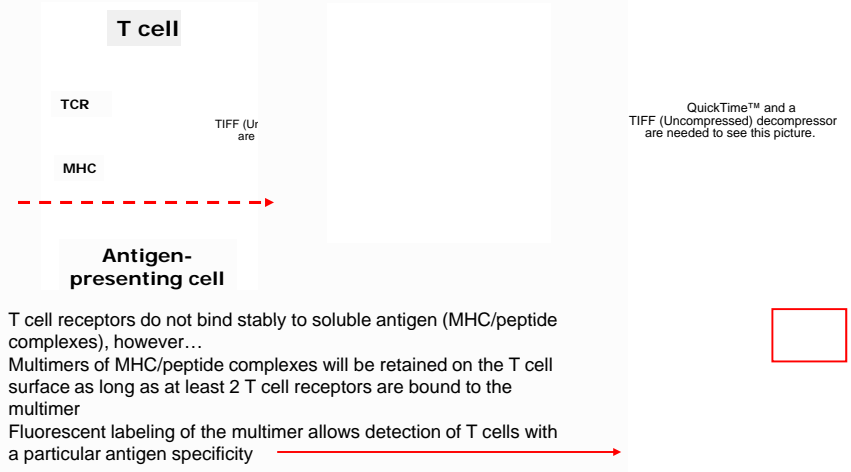
- **Research questions:**

- Do tumor-specific antibodies in the blood appear/increase after vaccine administration?
- Do tumor-specific T cells in the blood appear/increase after vaccine administration?
- Are tumor-specific T cells functional?

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Immune Monitoring: DC vaccine

MHC multimer (tetramer/pentamer) staining for antigen-specific

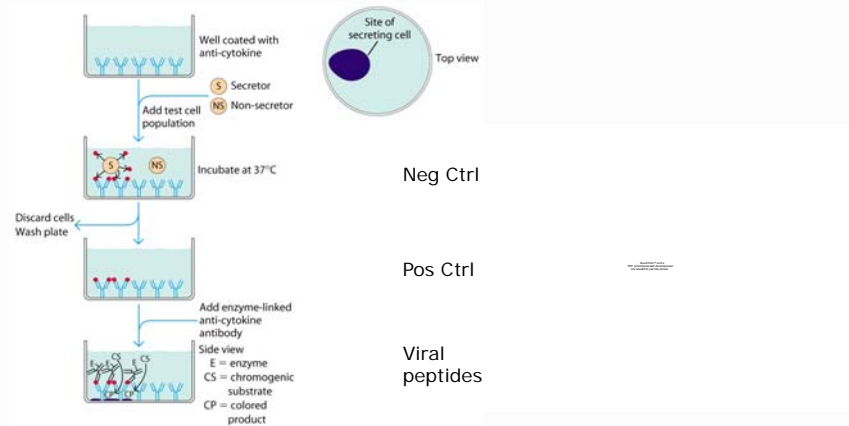


Adapted from Immunobiology, 5th Edition, C. Janeway et al, Garland Science

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Immune Monitoring: DC vaccine

ELISpot (Enzyme Linked Immunospot) Assay



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Immune Monitoring: DC vaccine

Sample	Whole blood phenotype	Freeze cells	Freeze serum
Baseline	X	X	X
Week 0	X	X	X
Week 4	X	X	X
Week 8	X	X	X
Week 24	X	X	X
Week 52	X	X	X

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Lessons Learned

- Planning early is critical, for example, to allow sufficient time for transfer of assay techniques to central lab
- Prioritize testing, for example, if sample size is limited, what testing is most important
- Review results at predetermined intervals, for example to confirm compliance with clinical protocol or to determine whether planned testing is informative and accurate
- Modify monitoring plan, e.g. in case planned assays are not providing answer to research questions or if unexpected results are found

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