Developing A Physiologically Based Pharmacokinetic Model for Quantitative Characterization of Exogenously Administered T Cells

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Interest in T cell based therapies

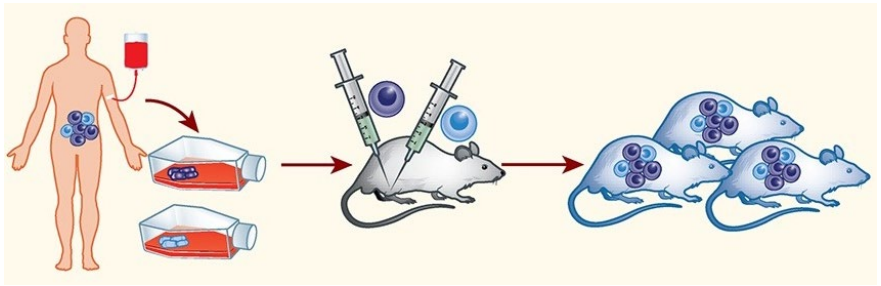
Adoptive cancer therapy

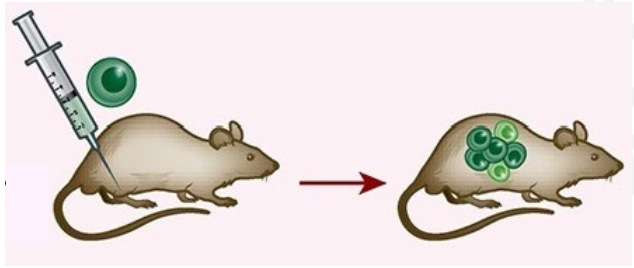
- TILs extracted from patients and activated ex-vivo with high IL-2
- Engineered T cells such as TCR T cells and CAR T cells

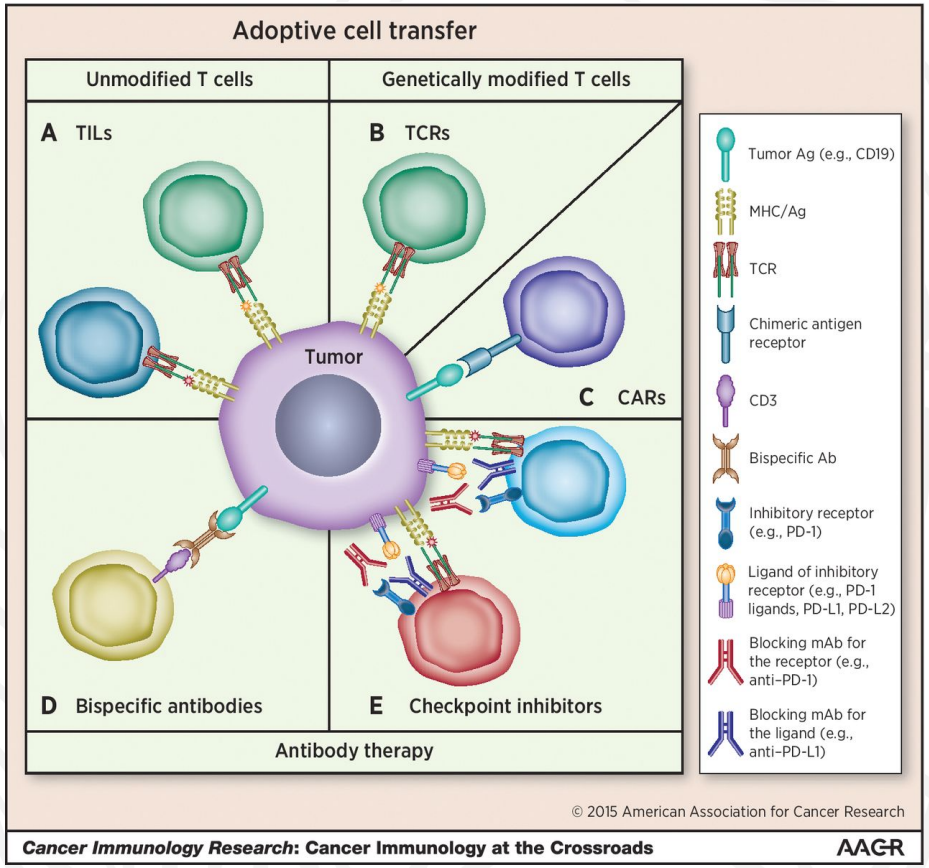
T cell redirecting/modulating therapies

- Immune check point inhibitors and bispecific antibodies

  - SCID mice + xenograft tumors + huPBMCs
  - WT mice + murine tumors + muTCR/CAR T cells or endo immune cells + IO therapy







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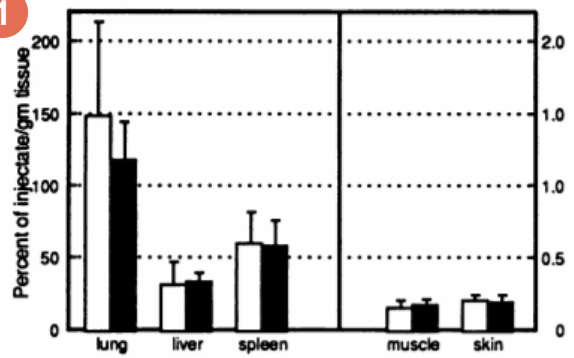
Buque, A. and L. Galluzzi (2018). "Modeling Tumor Immunology and Immunotherapy in Mice." Trends Cancer **4**(9): 599-601

Previous studies

ID	Authors (year)	Label used	State of T cells	Significant tissues
1	Wallace et al. 1993	[I125]-PKH95	Active T cells purified from tumor (antigen specific)	Lung (tumor metastases), spleen (in 20 hrs)
2	Albright et al. 1997	[I125]I2P-Di-6-ASP	Inactive, purified from spleen	Spleen, liver
3	Melder et al. 2002	In111 oxine	Tumor antigen specific activated T cells	Liver, spleen
4	Xu et al. 2013	CFSE dye	Tumor antigen specific activated T cells	Tumor, liver

1



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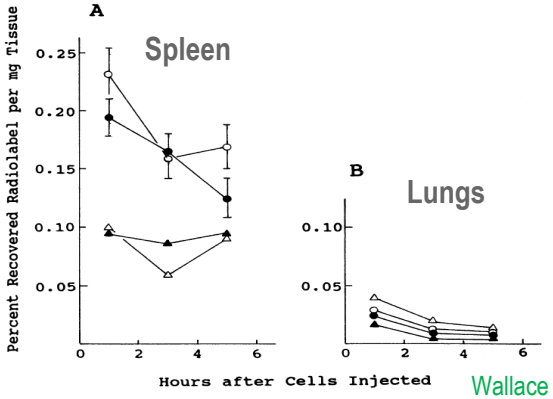

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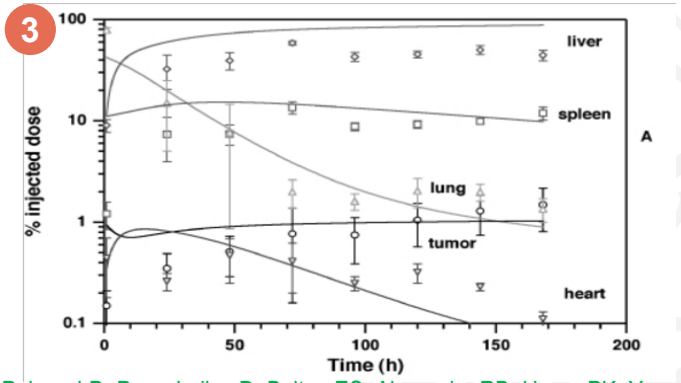

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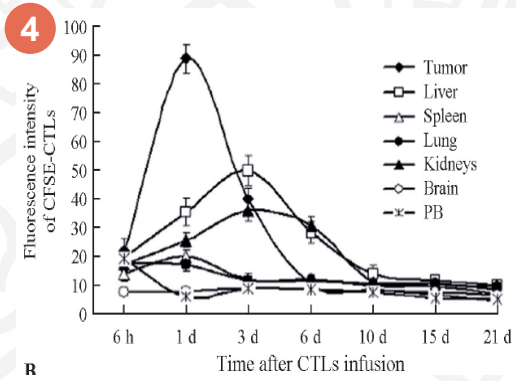


Wallace PK, Palmer LD, Perry-Lalley D, Bolton ES, Alexander RB, Horan PK, Yang JC, and Muirhead KA (1993) *Cancer Res* **53:**2358-2367.
Melder RJ, Munn LL, Stoll BR, Marecos EM, Baxter LT, Weissleder R, and Jain RK (2002) *Neoplasia* **4:**3-8.
Xu WL, Li SL, Wen M, Wen JY, Han J, Zhang HZ, Gao F, and Cai JH (2013) *Chin Med J (Engl)* **126:**3019-3025.
Albright, J. W., R. C. Mease, C. Lambert and J. F. Albright (1998). Mech Ageing Dev **101**(3): 197-211.

Use of chromium for labeling T cells

Method established 50 years ago…tried to see further by standing on the shoulders of giants

Chromium binds to intracellular peptides irreversibly. Chromium eluted from dead cells is not reutilized and is cleared out very fast. Half life of Cr51 – 27.7 days

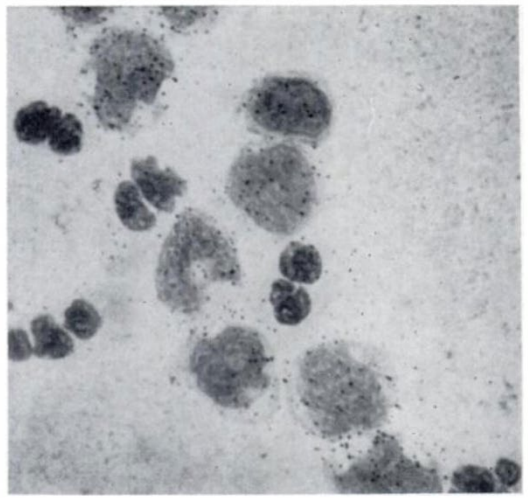


Fig. 1.—Representative 51-chromium radiograph of leukocyte suspension demonstrating heavier labeling of large lymphocytes and monocytes.

Lymphocytes showed higher labeling efficiency compared to erythrocytes

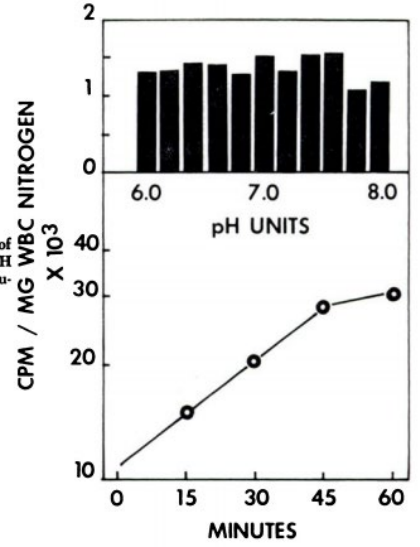


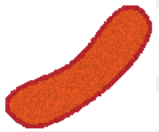
Fig. 1.—The relation of ^{51}Cr incorporation to pH and duration of incubation.

Optimized labeling conditions

Eyre HJ, Rosen PJ, Perry S (1970) Relative labeling of leukocytes, erythrocytes and platelets in human blood by 51-chromium. Blood 36 (2):250-253
McMillan R, Scott JL (1968) Leukocyte labeling with 51-Chromium. I. Technic and results in normal subjects. Blood 32 (5):738-754

Method of activating and labeling T cells

C57BL/6 mouse with melanoma



Spleen

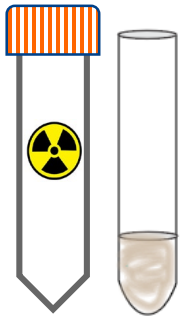
Splenocytes

Anti-CD28 mAb

Anti-CD3 mAb

IL-2

Activation and proliferation of splenocytes

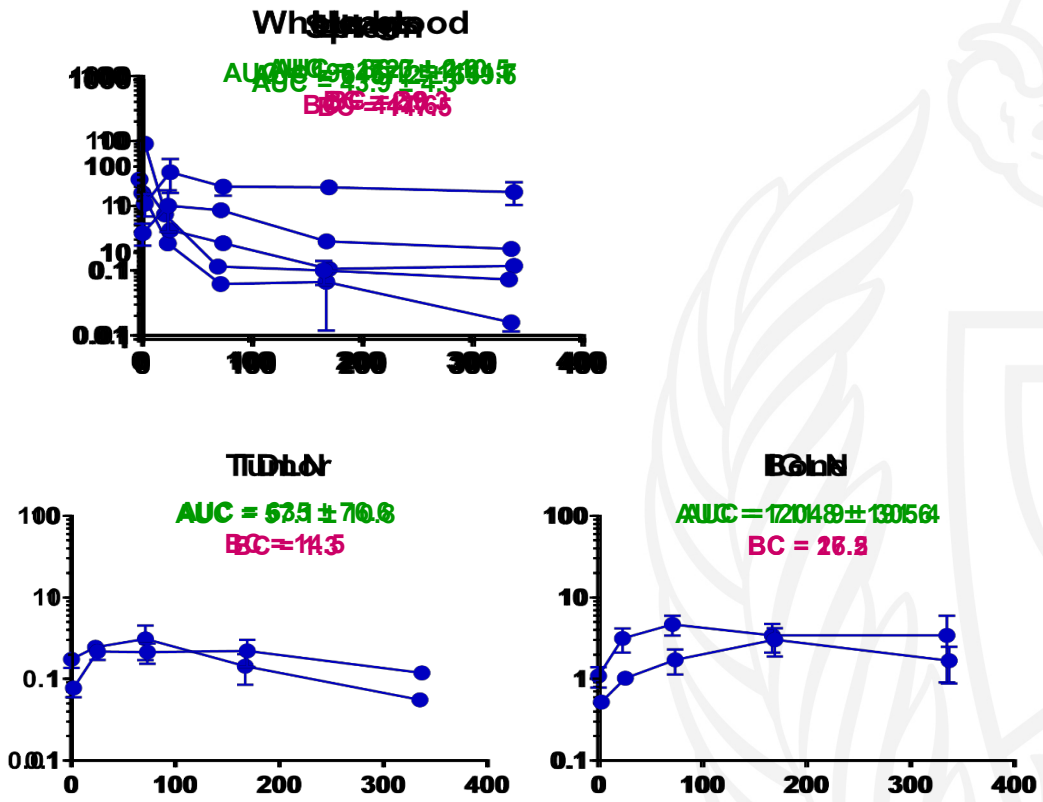


Labeling lymphocytes with Cr-51

Injecting labeled lymphocytes

Observed T cell PK and Biodistribution

AUC: area under the curve, %ID/g*hr ; BC: biodistribution coefficient, AUCtissue/AUCplasma



Y axis: %ID/g of tissue

X axis: Time, hours

Observed T cell PK and Biodistribution

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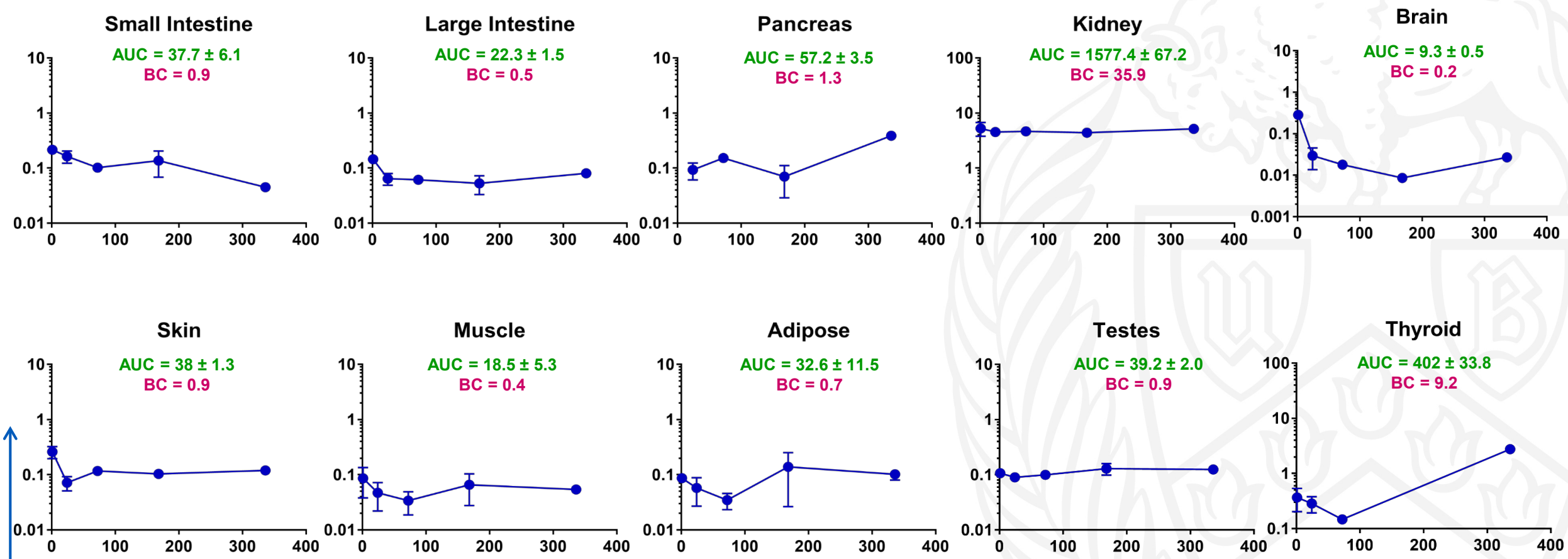
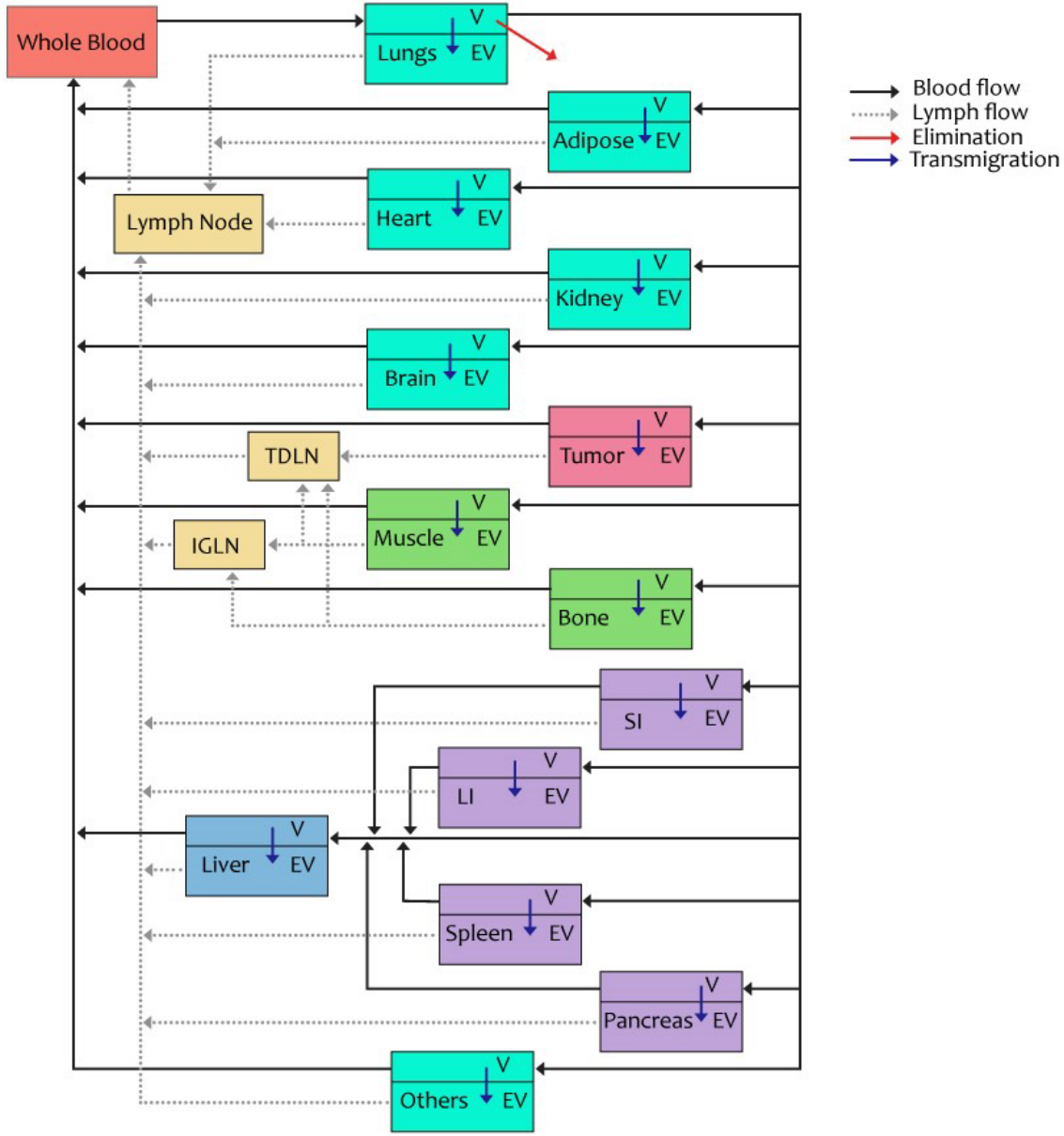




PBPK model

Model processes:

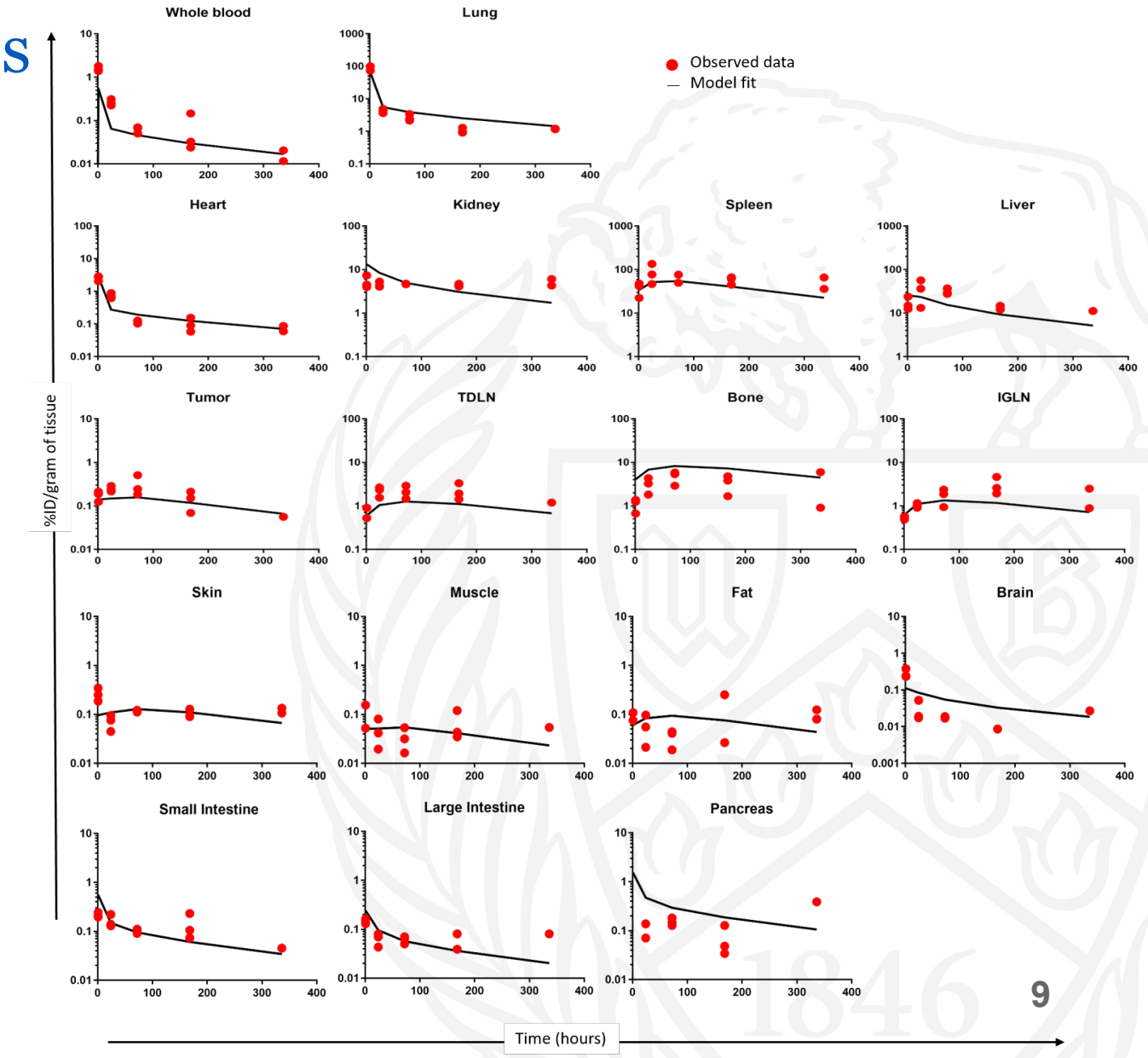
- Major tissues connected in anatomical manner
- Cells are distributed via blood flow
- T cells migrate into extravascular compartment of the tissue
- Recycling of T cells from extravascular compartment via lymph flow
- Lymphatic fluids drain into lymph nodes, which empties into whole blood compartment

Model assumptions:

- Elimination only through lung compartment
- All cells recycle from extravascular compartment
- Retention factor estimated for liver, spleen and kidney compartment to account for steady accumulation

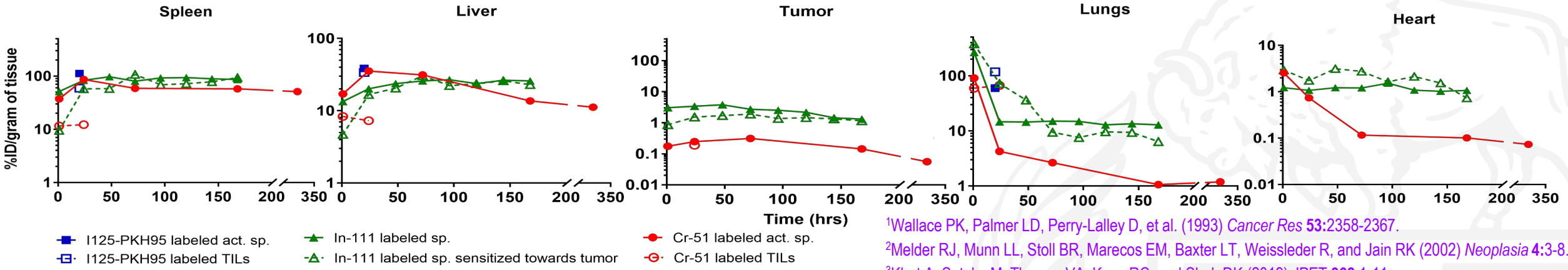
Model fits and parameter estimates

Parameter	Description (unit)	Estimate	CV%
J_{lung}	Transmigration rate for each tissue (1/hr)	1843.0	15.4
J_{heart}		34.9	17.2
J_{kidney}		87.3	37.9
J_{brain}		1.4	21.2
J_{muscle}		0.5	19.7
J_{bone}		82.6	16.7
J_{tumor}		0.6	18.6
J_{skin}		0.6	18.7
J_{fat}		1.6	18.5
J_{SI}		12.9	17.1
J_{LI}		5.2	18.1
J_{spleen}		114.0	33.8
J_{liver}		126.9	18.9
$J_{pancreas}$		10.0	20.0
J_{other}		86.8	17.7
R_{kidney}	Retention factor	3.9	37.9
R_{spleen}		9.8	34.8
R_{liver}		2.5	18.4
E_{lung}	Elimination rate (1/hr)	0.84	Fixed (Zhu et al., 1996)
Q_{tumor}	Tumor blood flow (ml/hr)	6	Fixed (Zhu et al., 1996)
V_{tumor}	Tumor volume (ml or g)	0.45	Fixed (radius~3.85mm)



Zhu H, Melder RJ, Baxter LT, and Jain RK (1996) *Cancer Res* **56**:3771-3781.

Comparison with literature reports



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[3]Khot A, Satoko M, Thomas VA, Koya RC, and Shah DK (2019) JPET **368**:1-11

Splenocytes exposed to 6000 IU/ml IL-2	TILs extracted from MC38 SC tumors
Spleen	**Lung**
Lung	**Spleen**
Liver	**Liver**
Skin	**Skin**
Muscle	**Muscle**

I-125 PKH95 labeled T cells[1]

Lymphocytes extracted from spleen	Sensitized with splenocytes of tumor-bearing mice and lymphocytes extracted from spleen
Spleen	**Lung**
Liver	**Spleen**
Lung	**Liver**
Tumor	**Tumor**
Heart	**Heart**

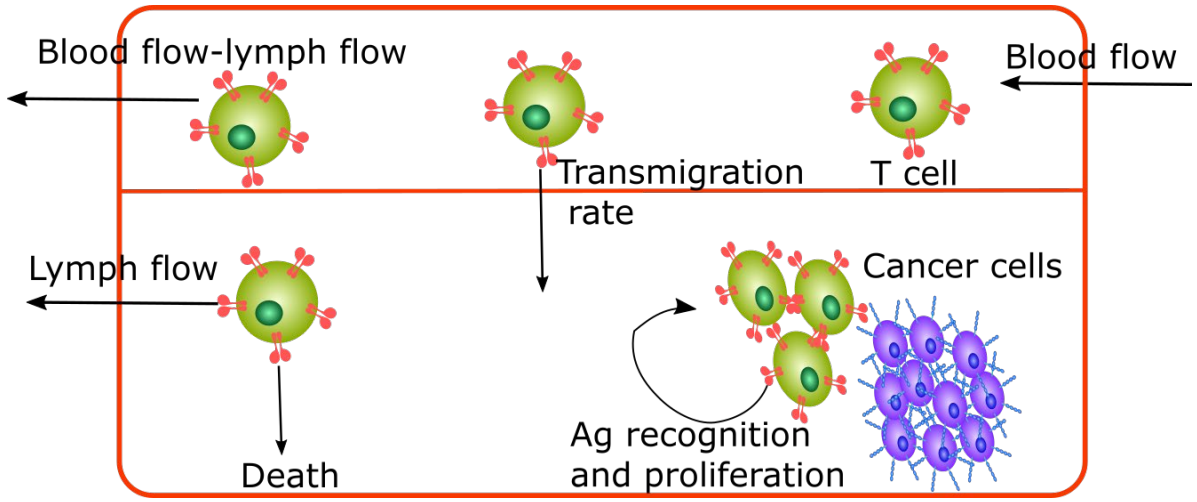
In-111 oxine labeled T cells[2]

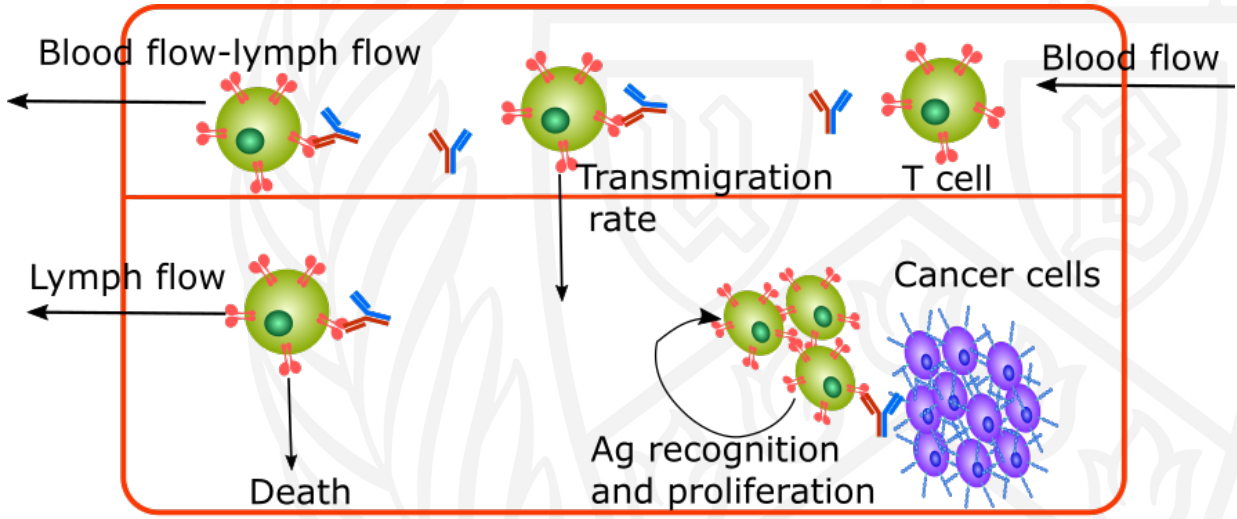
Splenocytes expanded using anti-CD3, anti-CD28 and IL-2	TILs extracted from B16-BL6 SC tumors
Spleen	**Lung**
Liver	**Spleen**
Lung	**Liver**
Kidney	**Kidney**
TDLN	**Tumor**

Cr-51 labeled T cells[3]

Future directions

- Foundation PBPK model -> predictive model by plugging experimental transmigration rates
- Use this method to investigate PK of specific cell types
- Expand this model to incorporate T cell antigen recognition and clonal proliferation
- Integrate this model with Ab PBPK model to describe the PK of T cell retargeting bispecific antibodies and predict in vivo synapse concentrations – NSG mouse model with huPBMC





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- Department of Pharmaceutics, SPPS, SUNY at Buffalo
- ASCPT 2019 scientific committee
- Pfizer Inc.

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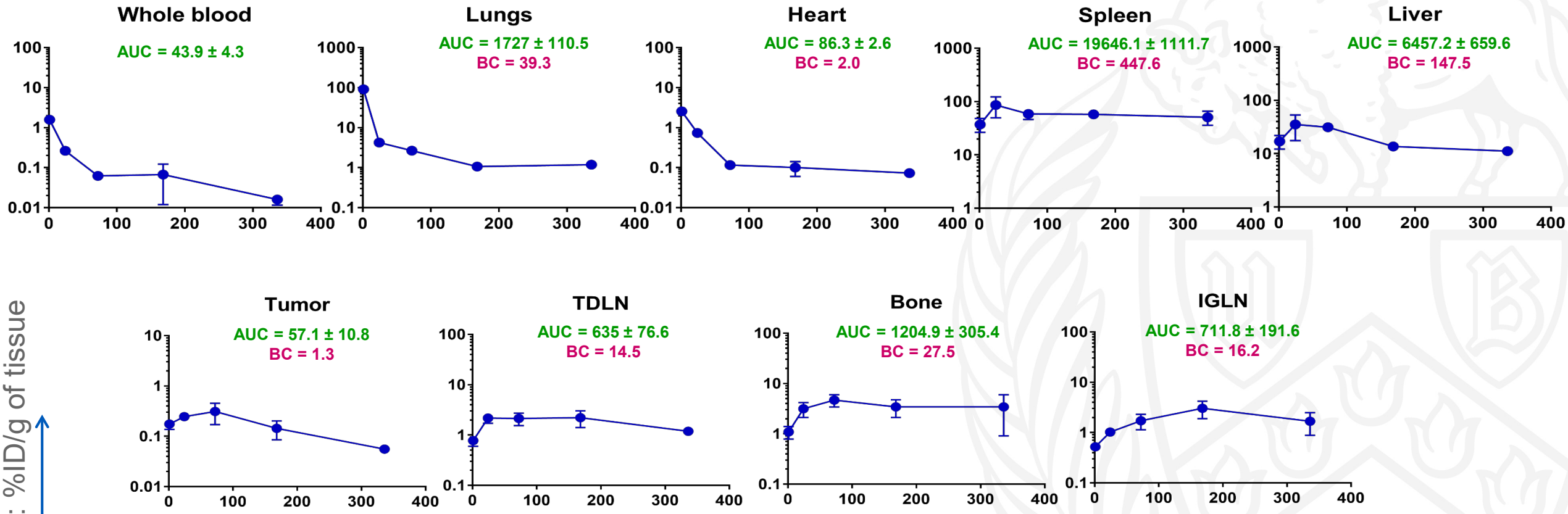

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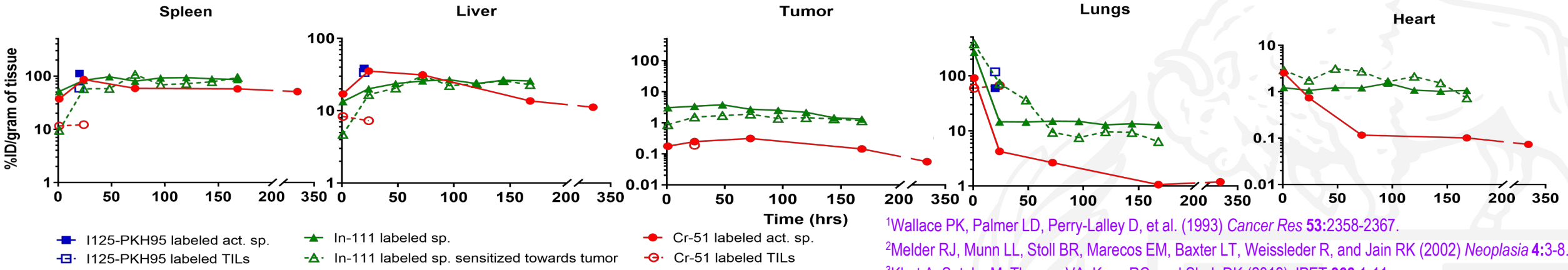

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Study ID	Wallace et al. (1993)		Melder et al. (2002)		Khot et al. (2019)	
Labeling method	I-125 PKH95 labeled T cells		In-111 oxine labeled T cells		Cr-51 labeled T cells	
Preparation of T cells	*Ag not recognized Splenocytes exposed to 6000 IU/ml IL-2	**Ag recognized TILs extracted from MC38 SC tumors	*Ag not recognized Lymphocytes extracted from spleen	Mice sensitized with splenocytes of MCaIV tumor-bearing C3H mice and lymphocytes extracted from spleen	Splenocytes expanded using anti-CD3, anti-CD28 and IL-2	TILs extracted from B16-BL6 SC tumors
Tissues listed in descending order of concentrations at 20-24 hours	Spleen	Lung	Spleen	Lung	Spleen	Lung
	Lung	Spleen	Liver	Spleen	Liver	Spleen
	Liver	Liver	Lung	Liver	Lung	Liver
	Skin	Skin	Tumor	Tumor	Kidney	Kidney
	Muscle	Muscle	Heart	Heart	TDLN	Tumor