# Positron emission tomography (PET) imaging in drug development, from bench to bedside application, with decision making capabilities


**Kevin Maresca**
Early Clinical Development –
Digital Science and Translational Imaging –
**PET / Molecular Imaging**


**ACS Annual Meeting, August 22nd, 2022**

# Digital Science & Translational Imaging - Vision and Impact

Provide end-to-end expertise in the emerging fields of translational imaging, wearable technologies, & continuous monitoring to improve clinical trials and increase the value of medicines for patients

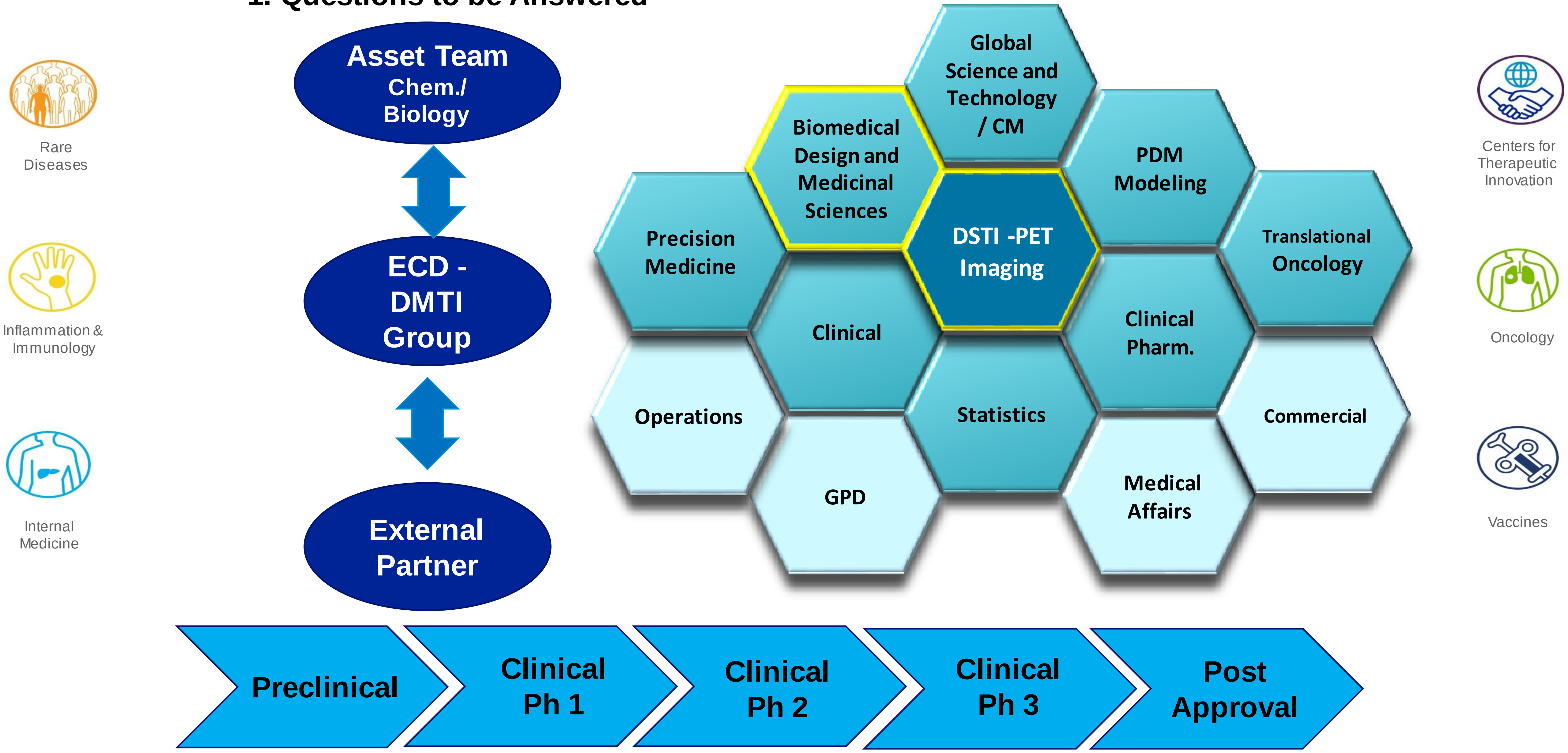
*Develop* novel digital and imaging endpoints relevant to patients

*Quantify* Proof of Mechanism & Signs of Clinical Activity

*Collaborate* across the enterprise

# Collaborative Matrix in Drug Development

## 1. Questions to be Answered



# Three Pillars of Survival in Drug Development



## Pillar 1: exposure at the site of action

- Does it get there?

## Pillar 2: binding to the pharmacological target

- Does it stick?

## Pillar 3: expression of pharmacology

- Does the drug do something?

*Morgan et al, Drug Discovery Today 2012;17:419-424*

# Expansion of PET Imaging in Trials

- \+ Highly accurate
- \- Invasive, painful, complications
- \- Limited frequency
- \- Limited tumor volume
- \- Sampling variability, poor repeatability,
- \- Reproducibility issues - Tumor heterogeneity
- \- Inter-observer variability
- \- Lengthened trial duration

**Biopsy**

**SOC Imaging (e. g. CT RECIST 1.1)**

- \+ Non-Invasive
- \+ Inexpensive
- \+ Broad nature of measurements
- \- Limited ability to detect early molecular changes
- \- Bulk tissue measurements / non-target based

***Molecular responses to therapy***

**PET Imaging**

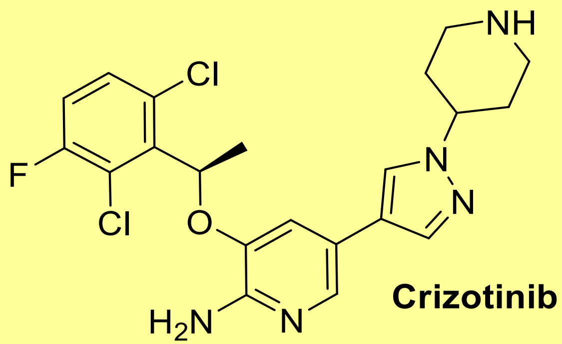
- \+ Non-Invasive
- \+ Highly sensitive / highly specific
- \+ Ability to detect earlier Tx responses
  - \+ **Proof of Mechanism**
- \+ Quantitative molecular measures of disease progression and response to therapy
  - ❖ **Need to Qualify Tracers to Progress**

# Pillar 1: Does the compound reach the target tissue? Introduction to Lorlatinib (PF-06463922)

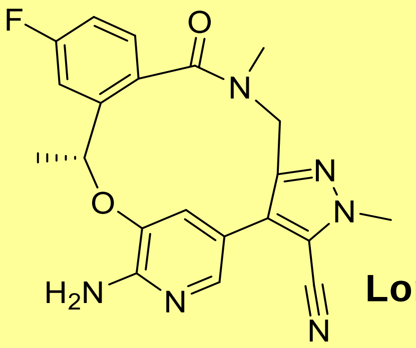
**Project: ALKi Lorlatinib – second generation to Crizotinib (approved in 2011 for locally advanced or metastatic NSCLC).**

- Protein kinase inhibitor that competitively binds within the ATP-binding pocket of target kinase
- Improved physical properties and selectivity.
- Improved potency for resistance mutations.
- Predicted to be brain penetrant (30% of NSCLC form brain mets)
- **Seeking enhanced brain permeability**

**Structures Crizotinib and Lorlatinib**




Crizotinib

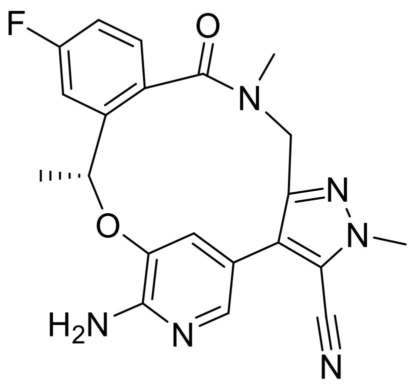



Lorlatinib

Pfizer

# Pfizer CNS PET Ligand Discovery Process

| Pharmacology | Low Non-specific Binding | Brain Permeability | Physicochemical Properties |
|---|---|---|---|
| Bmax/Kd > 10<br><br>> 30-100x Selectivity | Fu_b and Fu_p > 0.05, preferably Fu_p > 0.15<br>High risk of NSB if Fu_b = 0.05 and Fu_p = 0.05 | RRCK Papp AB > 5 x $10^{-6}$ cm/sec<br><br>MDR BA/AB ? 2.5 | CNS PET MPO > 3 |



**PF-06463922**

| | |
|---|---|
| **CNS MPO** | 4.5 |
| **CNS PET MPO** | 3 |
| | |
| **ClogP** | 1.86 |
| **ClogD** | 0.76 |
| **MWt** | 406 |
| **tPSA** | 110 |
| **HBDONOR** | 2 |
| **Pka** | 6 |

| | |
|---|---|
| **cRRCK** | 27.8 |
| **cMDR BA/AB** | 1.72 |
| **cFu_b** | 0.11 |
| **cFu_p** | 0.34 |

Zhang, L.; Villalobos, A. Strategies to facilitate the discovery of novel CNS PET ligands. *EJNNMI Radiopharm. Chem.* **2016,** 1:13.

Zhang, L. et al. J. Med. Chem. 2013, 56, 4568-4579.

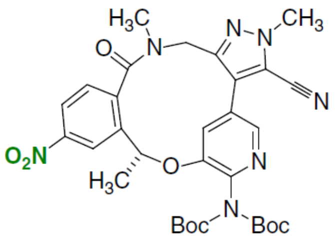
*$B_{max}$* and Bio-distribution → *Lead Triaging and PET-specific SAR* → *Assessing In vitro / vivo specific binding* → *PET imaging in NHP* → *GLP tox, dosimetry and eIND filing*

# Multi-pronged Strategy to Radiolabeling Lorlatinib

**Manual Synthesis:**

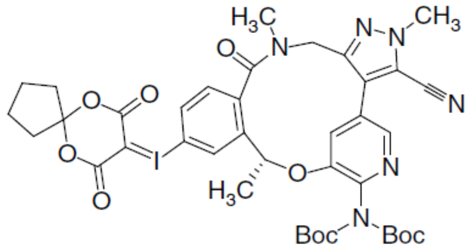
Employing ([18F] TEAF) following standard labelling procedures using Nitro precursor (3–4 mg) in DMSO and heated at 215 C for 15 min.

- Instability of compounds
- **<0.1% RCY**



Iodonium ylide-based radiochemistry **increased 14% RCY**

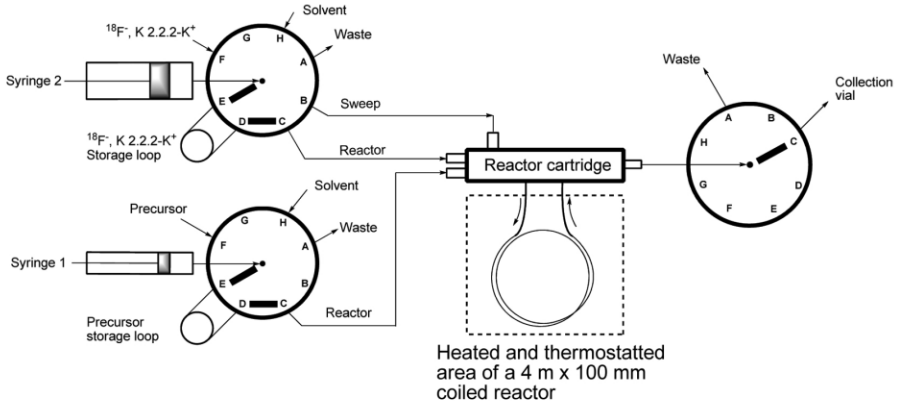
- Purification issues
- Cold residual issues



**Microfluidic Synthesis:**

Attempting to increase radiochemical conversion with challenging substrates (degradation or inability to use high temperature - employing di-Boc nitro precursor on a conventional system.

**RCY = only just over 1%**




**Loop method:**

Two-step methylation where the intermediate could be easily separated from the precursor, and could then be rapidly deprotected and purified

Simplicity (no heating or cooling required)

Ease of automation

Adaptation to the commercial radiosynthesis modules

**RCY = 3%**

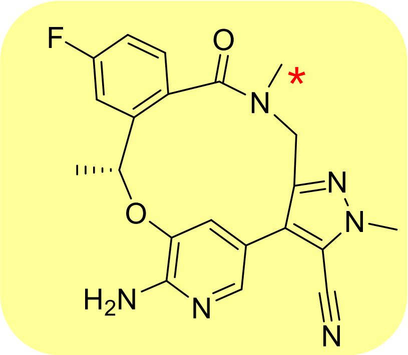
MGH/HST Athinoula A. Martinos Center for Biomedical Imaging

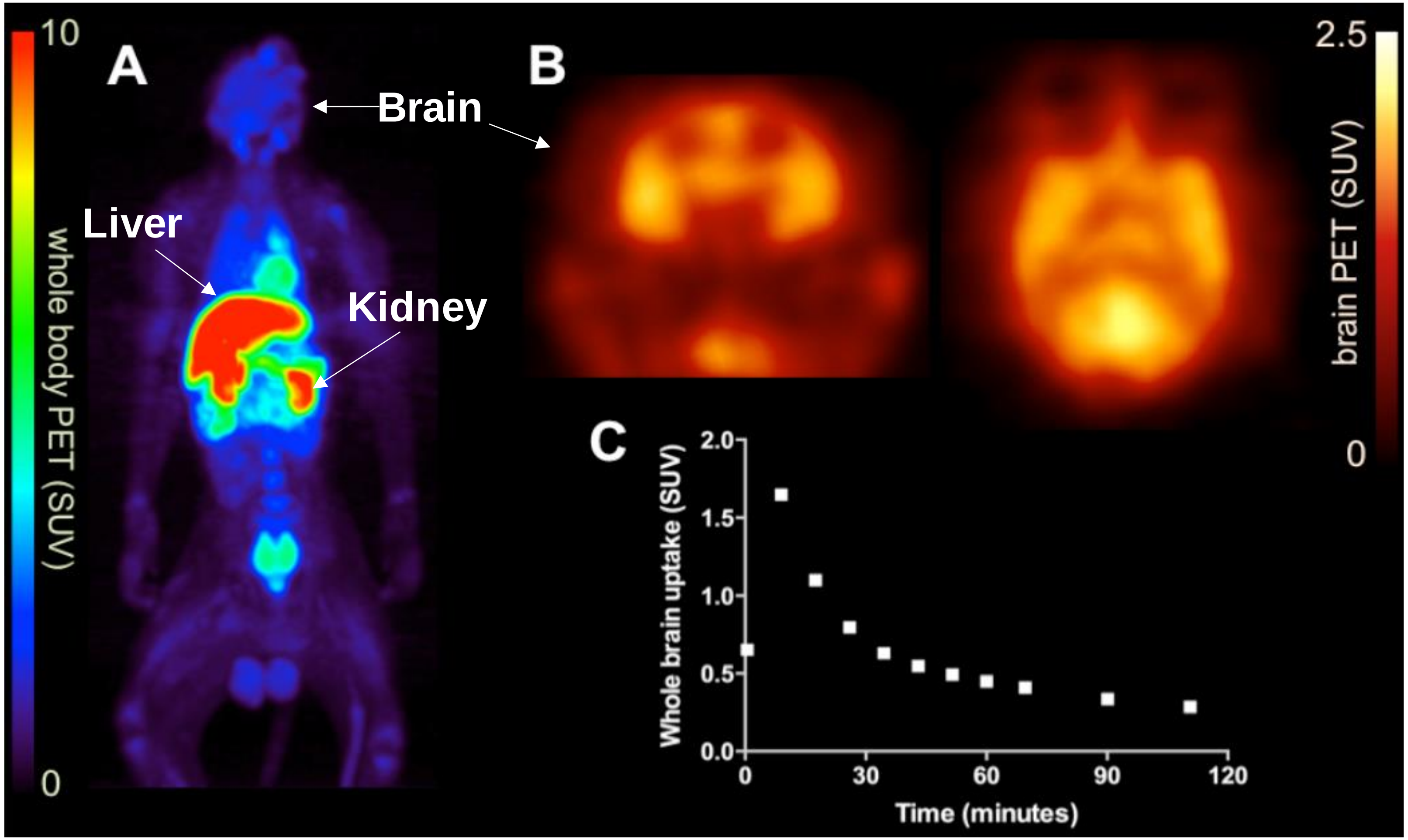
*Collier, T et al Nature Communications (2017), 8, 15761.*

# Pillar 1: Does the compound reach the target tissue?

**Non-Human Primate studies to evaluate the blood brain barrier penetration of the compound**

**Novel in-house PET Tracer**
Developed in collaboration with MGH

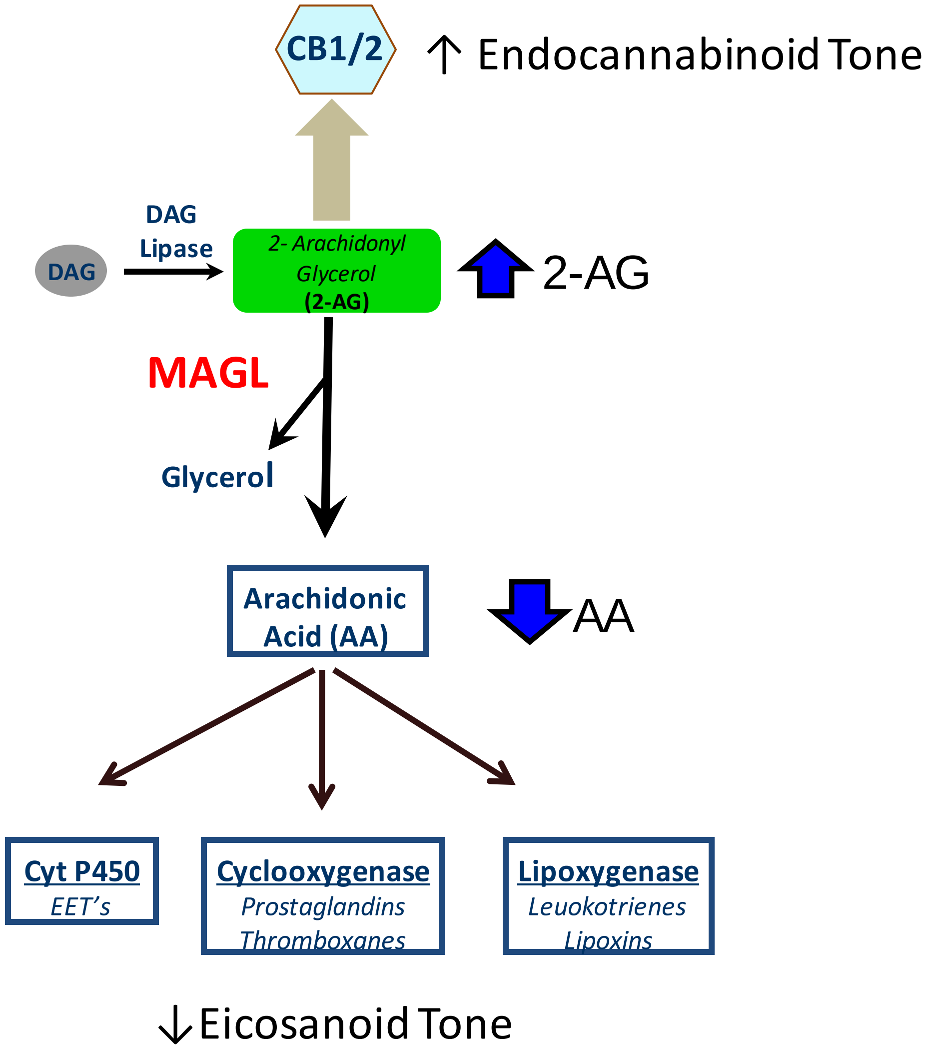



*Collier, T et al Nature Communications (2017), 8, 15761.*

# Pillar 2: Does the Drug Reach the Tissue and Bind to the Molecular Target?

- **Project Monoacylglycerol lipase (MAGL) as anti-inflammatory agent**
- Key **serine hydrolase** which terminates endocannabinoid signaling and **regulates arachidonic acid** driven **inflammatory responses** within the central nervous system




***1. Cannabinoid Pathway Activation***

***2. Pan-Arachidonate Cascade Interruption***

**MAGL Inhibition Presents a Novel Bi-directional Anti-inflammatory Strategy**

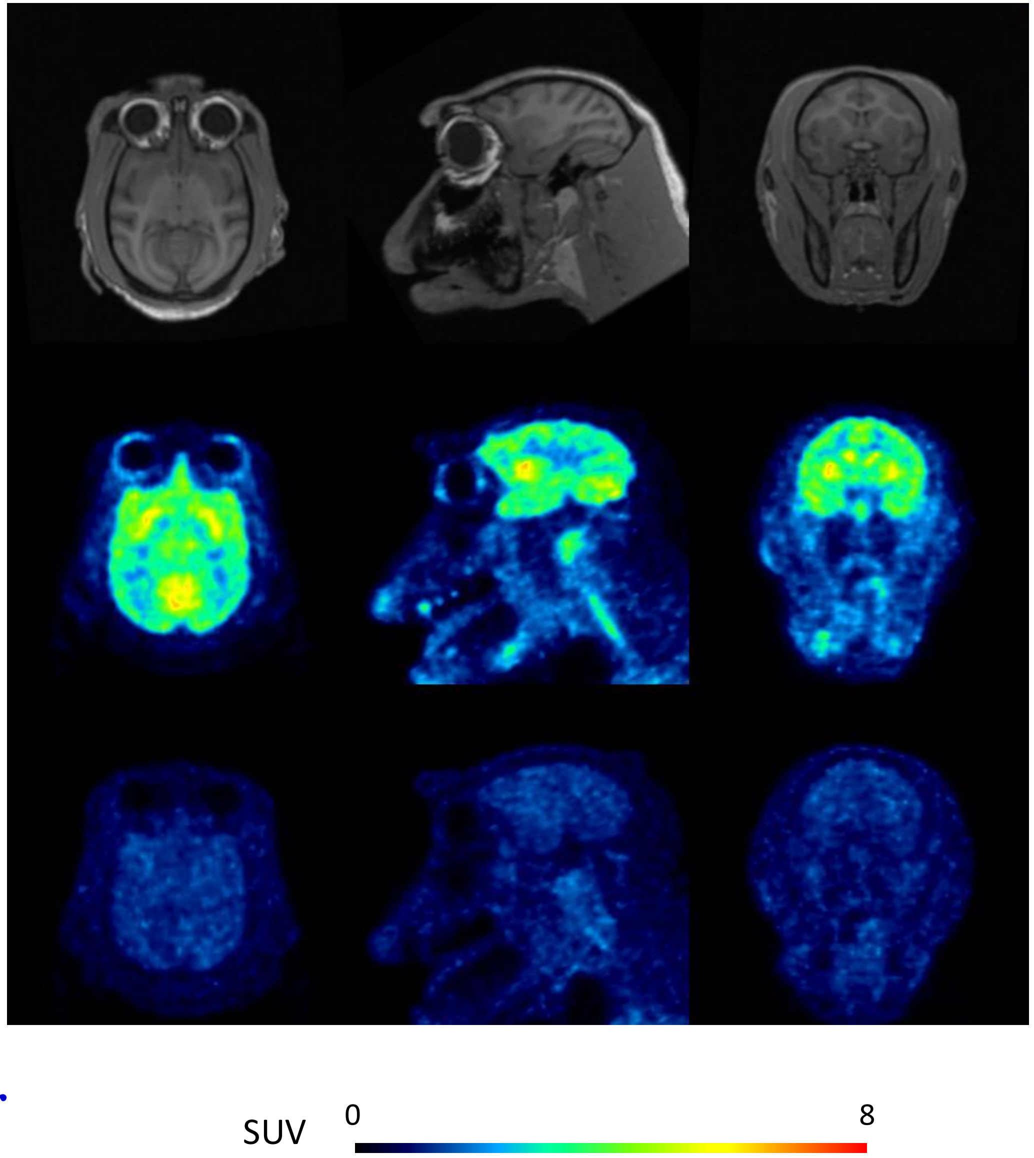
# Pillar 2: Does the Drug Reach the Tissue and Bind to the Molecular Target?

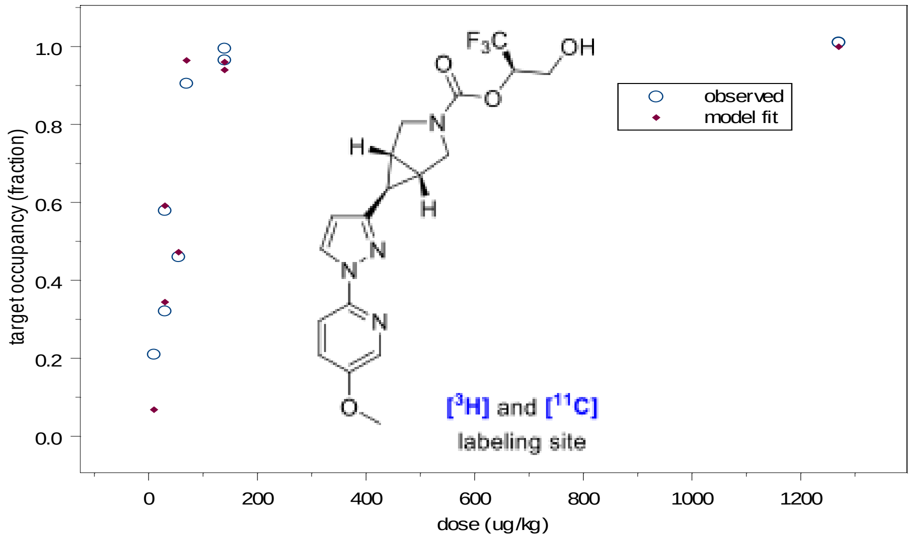
## MRI and PET summation images (all frames)

MRI

Baseline

Pretreatment




**High Brain Uptake**

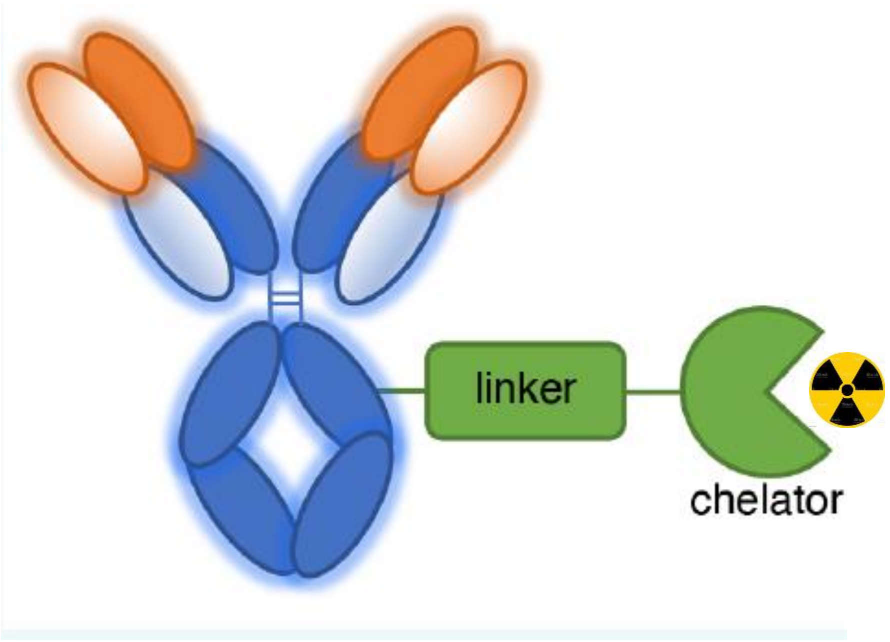
**Widely distributed**

**> 90% Blocking**

**Support of Clinical Dose Selections**

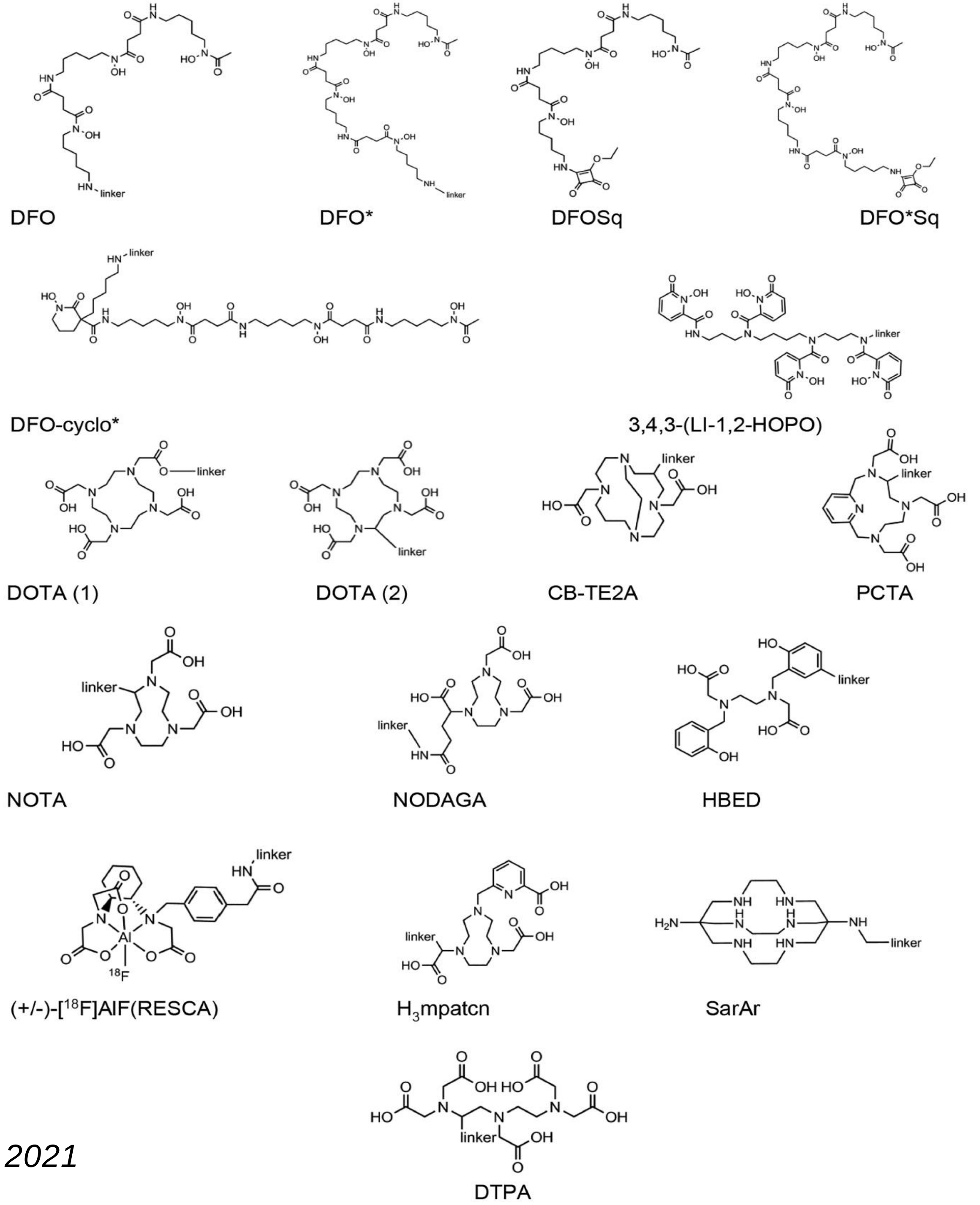
1. **Assessing / confirming the low dose is indeed subpharmacologic (Targeting <20% TO)**
2. **Assessing /confirming the top dose (AUC) has adequate coverage** * assuming 70 kg subject

# Antibody Interest-Growth of Metal-Based Radiolabeing




= radionuclide of choice

- **50:50 Split**
- **Fit for Purpose Chemistry**

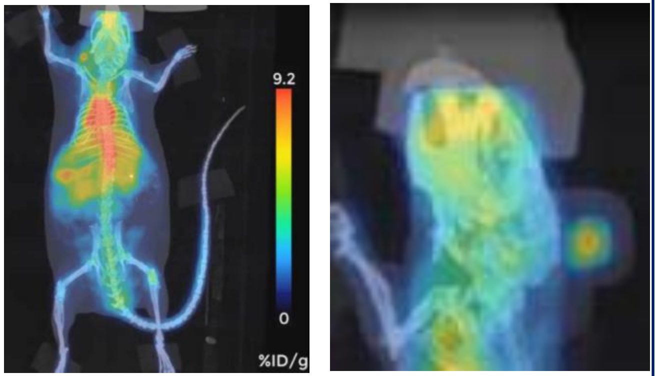


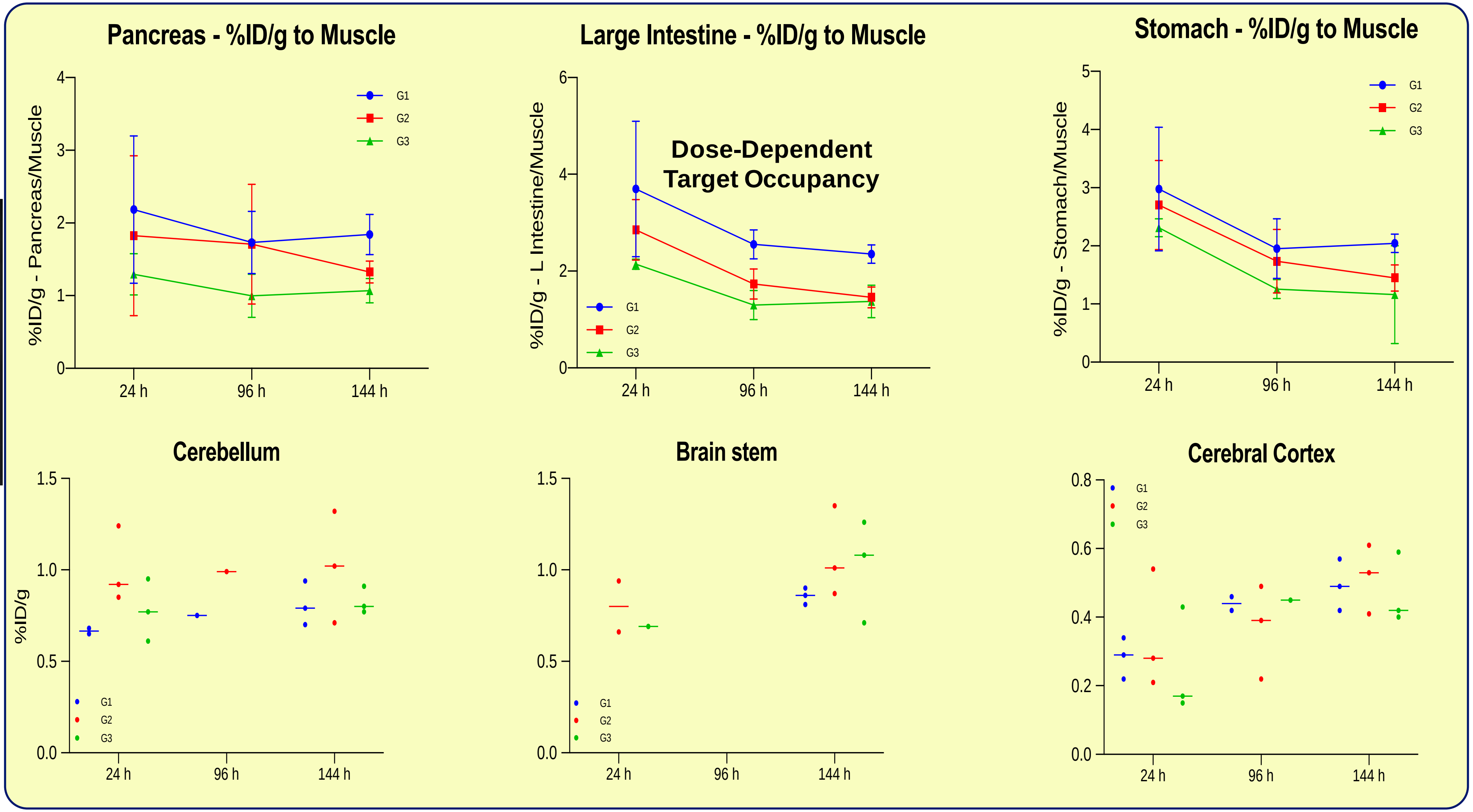

*Chomet and Vugts 2021*

Pfizer

# Question: Can PET imaging confirm Pillar 1 and Pillar 2 Assessments for Antibody

## Zr-89 Ab Imaging for Tissue Distribution and Occupancy



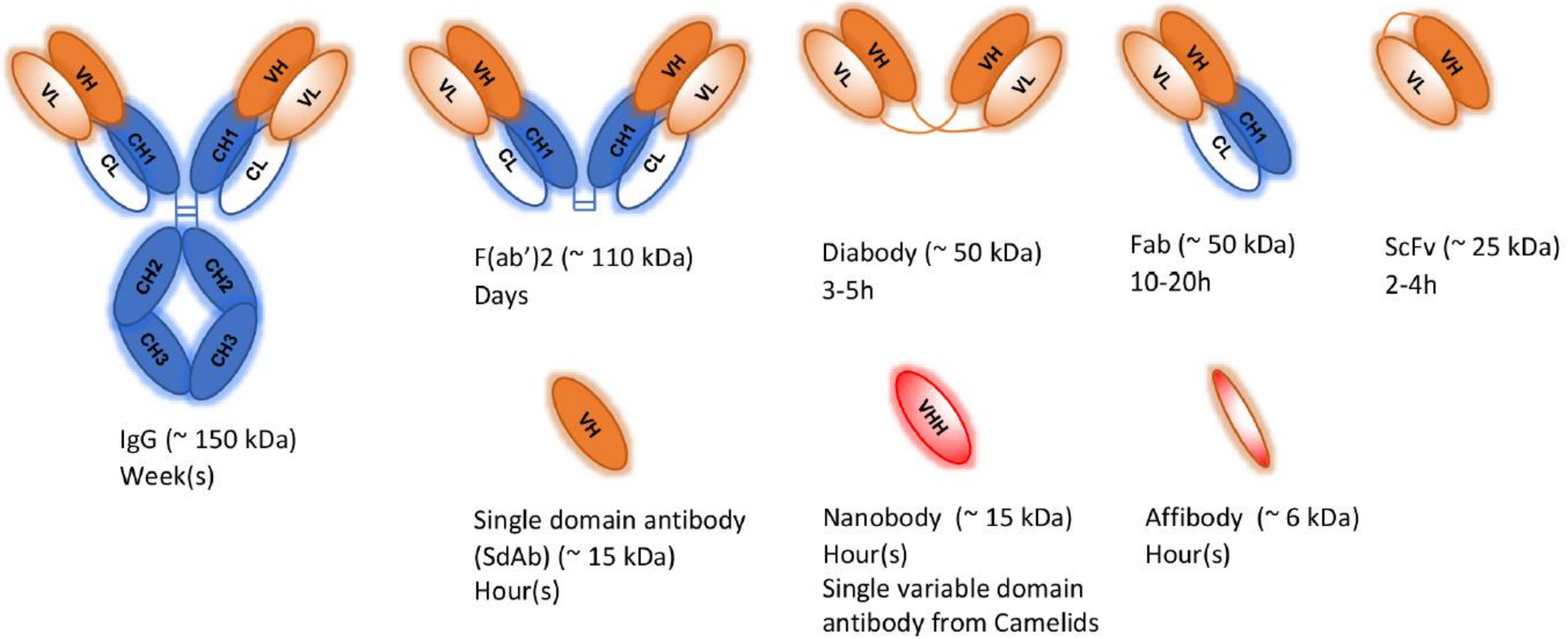




**Answer / Impact:** The imaging confirmed little/no brain penetration, and target occupancy was seen in various tissues, including the intestines

❖ **Timepoints elongated**

**Draft Data Courtesy of:**
*Ned Keliher*

# Antibody Truncation – Radiolabeling of Fragments




**Impact: Decreasing size while maintaining affinity allows for earlier more convenient imaging window**

*Chomet and Vugts 2021*

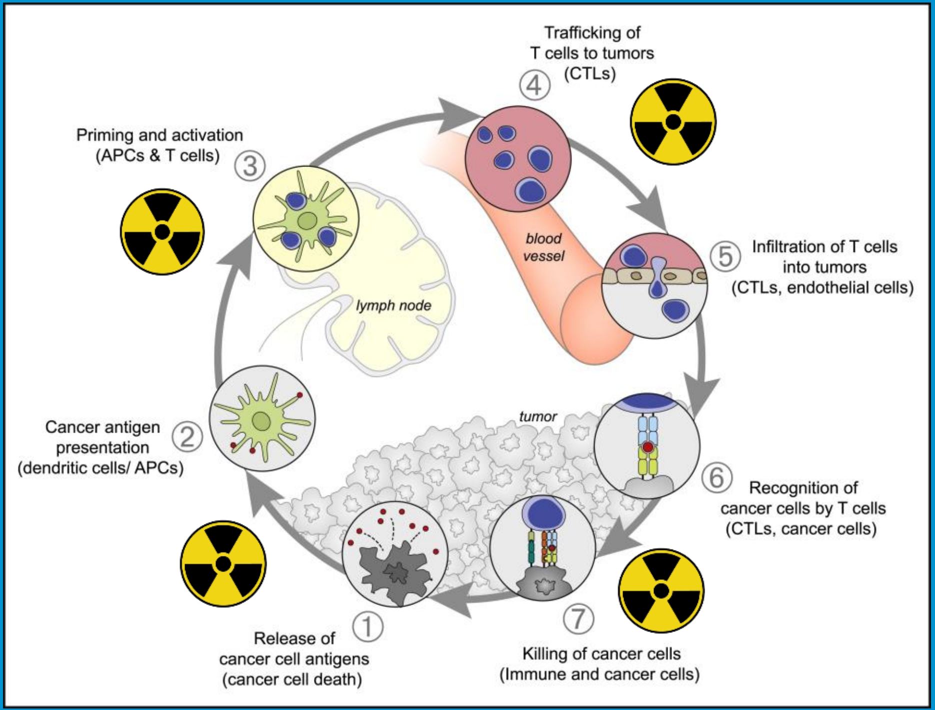
# Assessing PET Immune-Oncology (IO) Biomarkers

## The Value of PET IO Imaging

- ➢ All lesion Imaging (whole body)
- ➢ Imaging Directing Biopsy
- ➢ Ability to Identify Responders v. Non-responders
- ➢ Quantifiable (Quantitative Medicine)
- ➢ Clinically translatable

- ❖ Early Go / No-Go Decisions on Extensive Pipeline of Drugs / Combos?
- ❖ Earlier and more accurate Molecular Measure of progression?

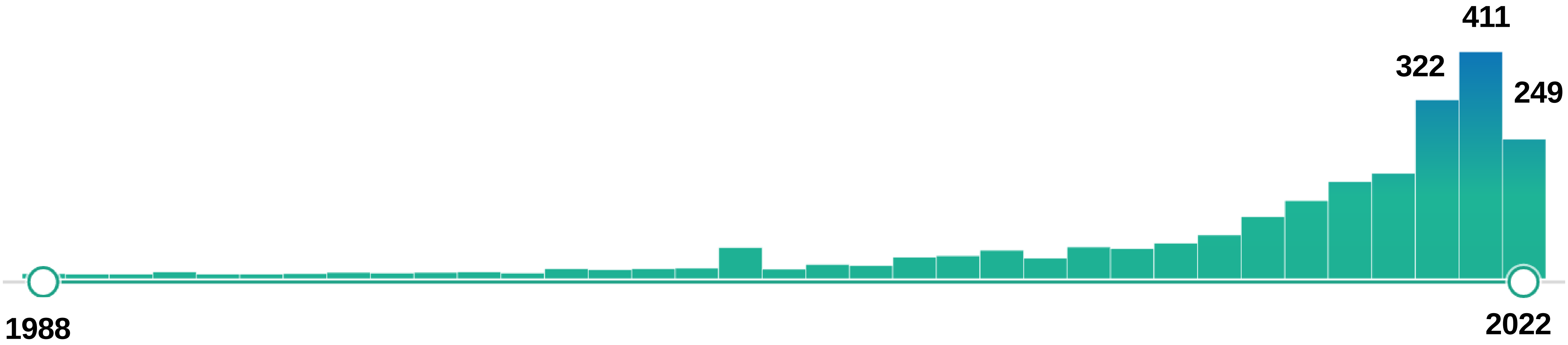
**Visualizing the Immune System Response with PET Imaging**




*Adapted from Daniel S. Chen - Immunity 39, 2013*

**Potential to be Transformative for PD monitoring**

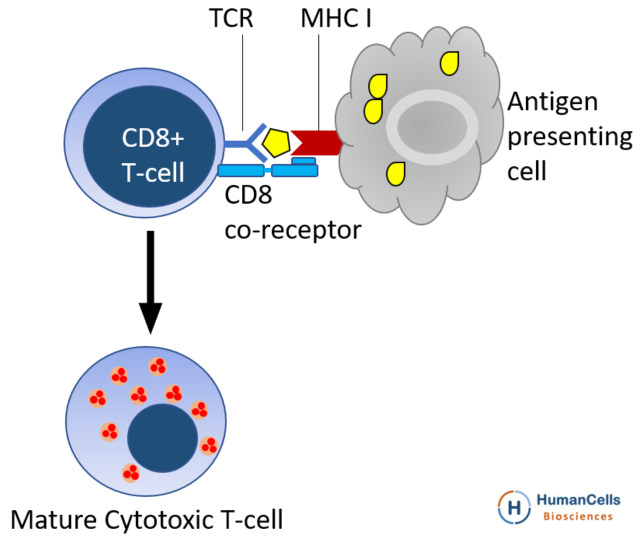
# Emerging Field – “PET Immune Imaging”




PubMed Search August 2022 on “PET Immune Imaging”

# Importance of CD8 T cells

- 10 publications in 2021
- >200 total
- Ab, Mb, Nb,
- Cu 64, Zr-89, F-18




*Research Article*

**PET Imaging of CD8 via SMART for Monitoring the Immunotherapy Response**

Lingyi Sun[1], Zhonghan Li[1], Yongyong Ma[1], Johannes Ludwig[2], Hyun S. Kim[3,4] and Dexing Zeng[1,5]

scientific reports

OPEN

**Positron emission tomography imaging with $^{89}$Zr-labeled anti-CD8 cys-diabody reveals CD8⁺ cell infiltration during oncolytic virus therapy in a glioma murine model**

Benjamin B. Kasten[1,9], Hailey A. Houson[2,9], Jennifer M. Coleman[1], Jianmei W. Leavenworth[1,3], James M. Markert[1,3], Anna M. Wu[4,5], Felix Salazar[5], Richard Tavaré[6], Adriana V. F. Massicano[2], G. Yancey Gillespie[1,3], Suzanne E. Lapi[2,3], Jason M. Warram[3,7] & Anna G. Sorace[2,3,8]

Zhao et al. J Nanobiotechnol (2021) 19:42
https://doi.org/10.1186/s12951-021-00785-9
Journal of Nanobiotechnology
RESEARCH Open Access

**ImmunoPET imaging of human CD8⁺ T cells with novel $^{68}$Ga-labeled nanobody companion diagnostic agents**

Haitao Zhao[1†], Chao Wang[2†], Yanling Yang[2,3], Yan Sun[2], Weijun Wei[1,4], Cheng Wang[1], Liangrong Wan[1], Cheng Zhu[1], Lianghua Li[1], Gang Huang[1,5] and Jianjun Liu[1,4*]

CLINICAL CANCER RESEARCH | PRECISION MEDICINE AND IMAGING

**Imaging Tumor-Infiltrating Lymphocytes in Brain Tumors with [$^{64}$Cu]Cu-NOTA-anti-CD8 PET**

Veronica L. Nagle[1], Kelly E. Henry[2], Charli Ann J. Hertz[3], Maya S. Graham[4,5], Carl Campos[3], Luis F. Parada[4,6], Neeta Pandit-Taskar[2,7,8], Andrea Schietinger[9], Ingo K. Mellinghoff[1,3,4,5], and Jason S. Lewis[1,2,7,10,11]

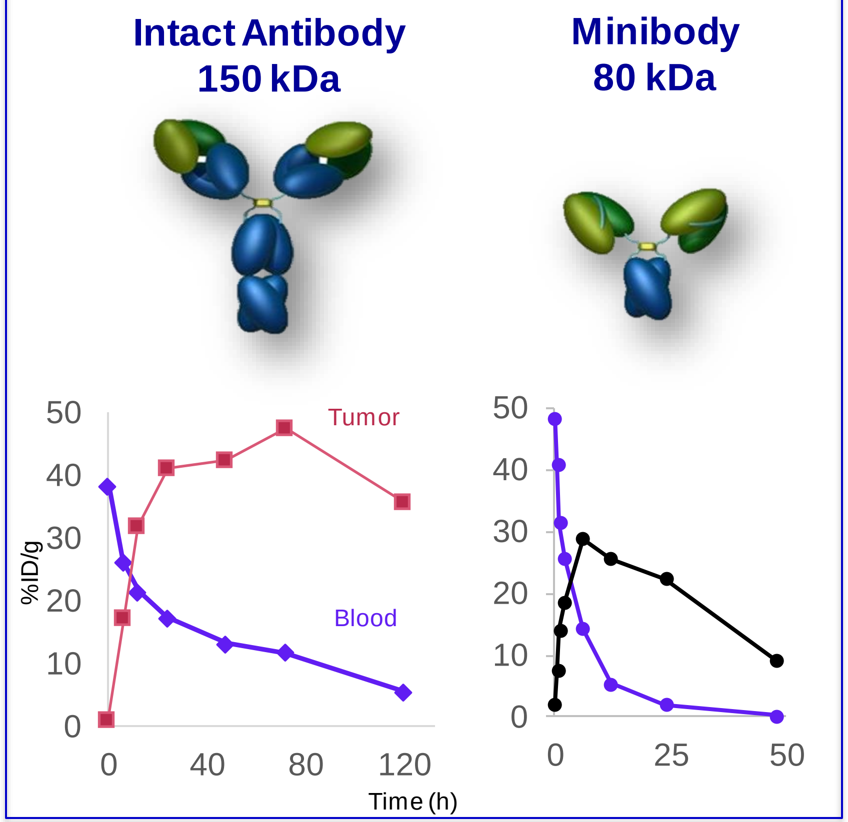
CANCER RESEARCH | TUMOR BIOLOGY AND IMMUNOLOGY

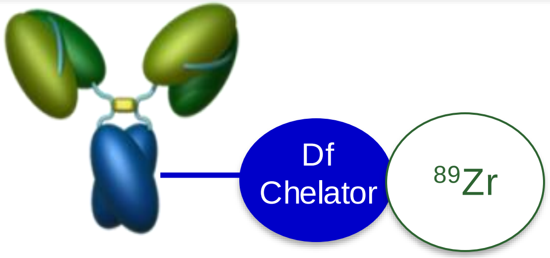
**The PET-Tracer $^{89}$Zr-Df-IAB22M2C Enables Monitoring of Intratumoral CD8 T-cell Infiltrates in Tumor-Bearing Humanized Mice after T-cell Bispecific Antibody Treatment**

Christoph M. Griessinger[1], Tove Olafsen[2], Alessandro Mascioni[2], Ziyue Karen Jiang[2], Charles Zamilpa[2], Fang Jia[2], Michael Torgov[2], Jason M. Romero[2], Filippo Marchioni[2], Daulet Satpayev[2], Chenyu Lee[2], Green Zhang[2], Tapan K. Nayak[1], Mudita Pincha[3], Maria Amann[3], Preethi L.B. Mohan[3], Marine Richard[3], Valeria G. Nicolini[3], Johannes Sam[3], Christina Claus[3], Claudia Ferrara[3], Peter Brünker[3], Marina Bacac[3], Pablo Umana[3], Dominik Rüttinger[4], Ian A. Wilson[2], Jean Gudas[2], Christian Klein[3], and Jean J.L. Tessier[1]

# Introduction to CD8 ImmunoPET: $^{89}$Zr-Df-Crefmirlimab

**Minibody platform** provides PET agents optimized for rapid clinical imaging




- Humanized engineered antibody. Around half the size of an intact antibody (“minibody”).
- Lack of effector functions (complement binding, ADCC)
- High affinity for human CD8 cells (with 0.4 nM affinity)
- $^{89}$Zr. 3.2 day ½ life.
- Same or next day imaging (vs 7-10 day for intact antibody)
- $T_{1/2}$ reduction for accelerated clearance to liver or kidney
- Repeat imaging potential
- Platform clinically validated

*“ImmunoPET marries positron emission tomography (PET), a technique that uses radioactive tracers to visualize the functions of human tissues, to an antibody’s propensity to home in on the cells it’s made to recognize.”*
*Nature, Volume 543, pg. 743*

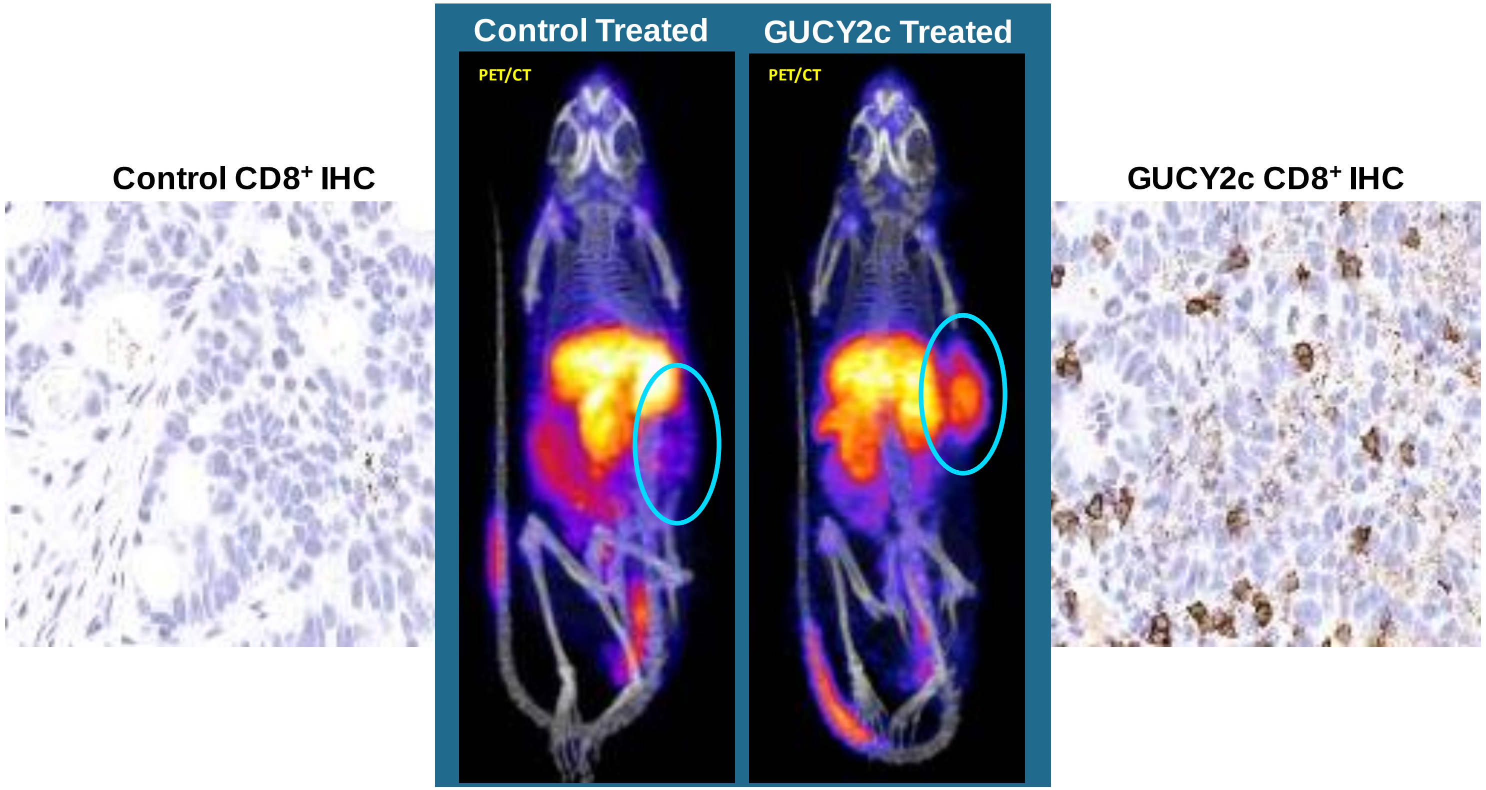
Pfizer

ImaginAb

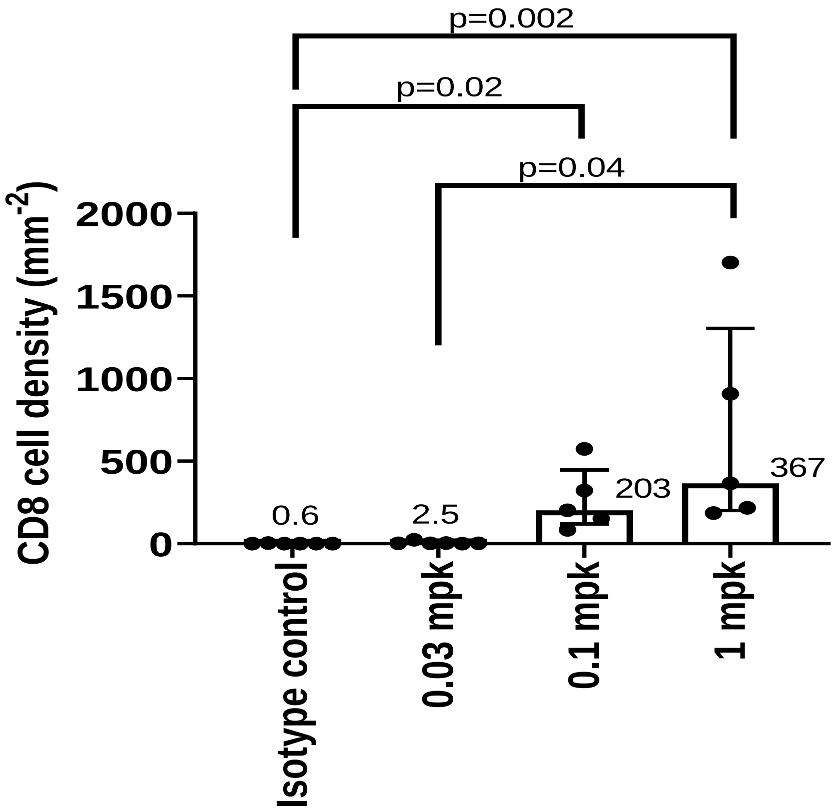
# Pillar 3: Does it affect downstream pharmacology?

**Project: GUCY2C CD3 Bispecific as an Immunotherapeutic treatment**

**Can we image CD8 T cells and their recruitment into tumors?**



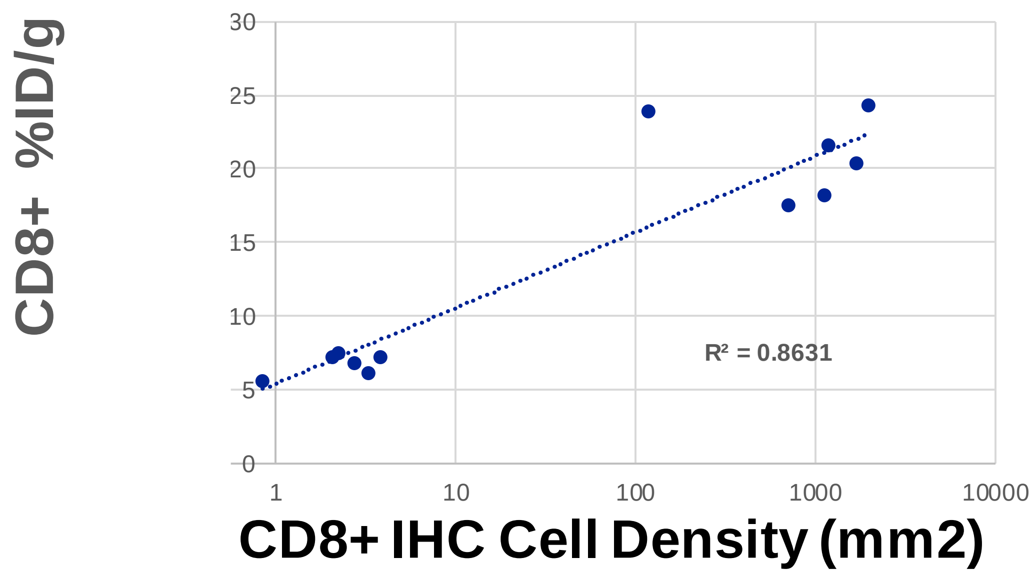

[$^{89}$Zr]CD8 PET in tumors



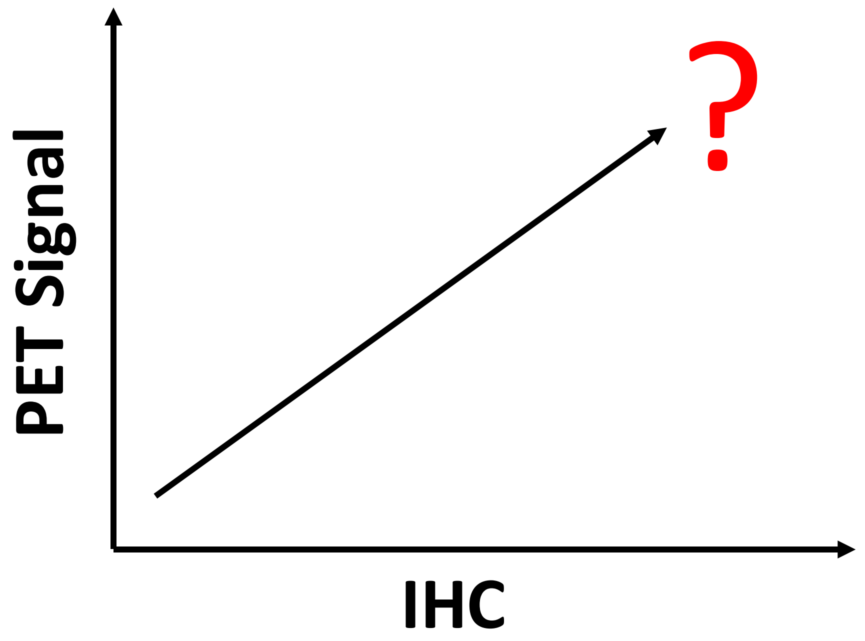

*Maresca K and Chen J., et al Submitted SNMMI (2020).*

# Orthogonal Measures are Key for Qualification

## Preclinical Qualification




## Clinical Qualification



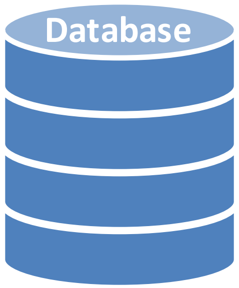

**Comparison to Ground Truth as Biomarker Evaluation/ Validation Continues to Define Future use**

# Pre-competitive Consortium with ImaginAb: $^{89}$Zr-CD8 Minibody PET Imaging

## CD8 Collaborative Group

ImaginAb

- Manufacturing
- Regulatory
- Site audits and training
- Trial sponsor
- Day to day execution




- Full access for pharma members
- Continually updated
- Reference data for IOTs in development

Joint Steering Committee


- Guidance for trial design (choice of tumor types, IOT, etc.)
- Guidance for standardization of image acquisition, data post-processing, data analysis
- Program oversight
- Approves funding beyond initial commitment

Pfizer AstraZeneca Takeda ImaginAb

# Melanoma Pt on Pembro – CD8 PET Response - Faster than SOC CT

**CD8 PET Pre-TX**

4 weeks
Keytruda

**CD8 PET**
**~ 1 mo Post-TX**

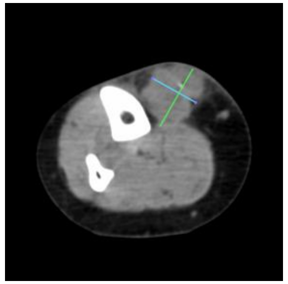
**CD8 PET Pre-TX**

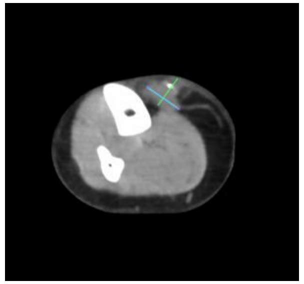
**CD8 PET**
**~ 1 mo Post-TX**

- ❖ **Early Avid CD8 PET uptake at 1mo**
- ❖ **Reduced Tumor Size at 4mo**

**CT**

| | **37mm x 28 mm** | **24 mm x 20mm (-35% :PR)** |
|---|---|---|
| | **Pre-TX** | **~ 4mo Post-** |





Pfizer AstraZeneca Takeda ImaginAb

# CD8 PET Imaging: Early IO Phase - Potential Decision Tree

**CD8 PET/CT Baseline Scan**

Pfizer IO Therapy ~4W

**CD8 PET/CT Treatment Scan**

If Most Pts are:

| CD8 PET Hot & CT Positive | CD8 PET Cold & CT Positive | CD8 PET Hot CT Negative | CD8 PET Cold CT Negative |
|---|---|---|---|

Then:

| Proceed as Monotherapy | Proceed as Monotherapy, Investigate timing of immune infiltration | Combine with another agent before Proceeding | No Go |
|---|---|---|---|

*Maresca 2021*

# Application of $^{89}$Zr-Df-Crefmirlimab in Early IO Therapy Programs

## Aim 1: Data w/ Pfizer Assets - Confirm Previous Results

- Early Proof of Mechanism (PoM) linked directly to Mechanism of Action
- Generates library of PET data to establish confidence
- Guidelines for future study design and building objective decision rules

## Aim 2: Decision-making Use for early Proof of Mechanism

- Definitive PoM 8 weeks prior to mature RECIST data -- Potential for early declaration of PoM
- No-Go Tool – Early termination of dose expansion cohorts
- Saving $XM+ for each stopped program moving forward

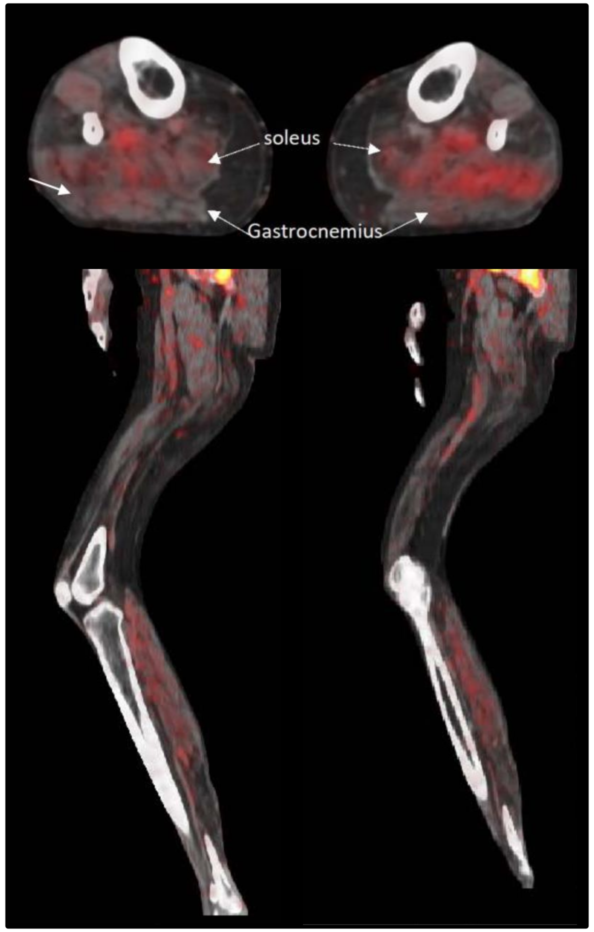
**Earlier Decision Enabling Tool**

# CD8 Mb PET Expands From Oncology Setting : Inclusion Body Myositis (IBM)

IBM Patient

Inclusion body myositis is **a rare inflammatory condition that causes muscle weakness and damage.**

Diagnosing IBM can be challenging because the symptoms are not unique to this condition.




**<u>Compared to Oncology</u>**

**Similar uptake:**
Lymph Nodes
Lung
Liver
Spleen
Kidney
Heart

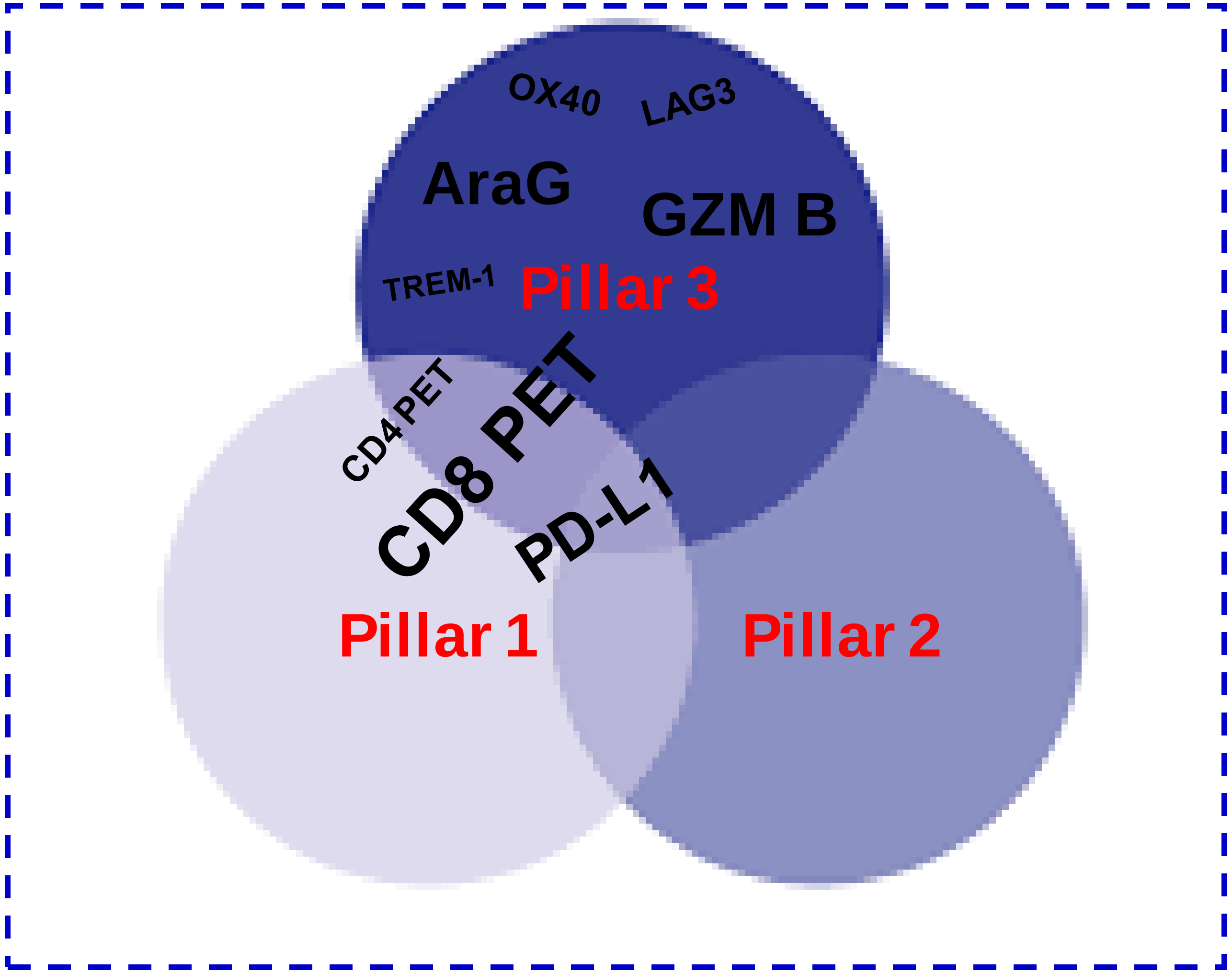
**Dissimilar:**
Muscle
Bone Marrow

ImaginAb

Penn
University of Pennsylvania

Images courtesy of:
1. Colin Quinn & Kelsey Moulton, Department of Neurology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA
2. Michael Farwell, Department of Radiology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA

# Three Pillars in PET Imaging in Trials




## <u>Tracer Selection</u>

- ***Depends on the Asset Question***
- ***Broadest Target Indication***
- ***Maximum Return on Investment***
- ***Alignment of Timing and Willingness***

# Our Plan: Short Term v. Long Term Strategies

**Balance of Short term → Long term strategies**

•**Short Term:** Assess signal, $\Delta$ signal , sensitivity, optimal imaging times in "validated" preclinical models

•**Long Term:** Project based / model based→ Go / No-Go Clinical decisions

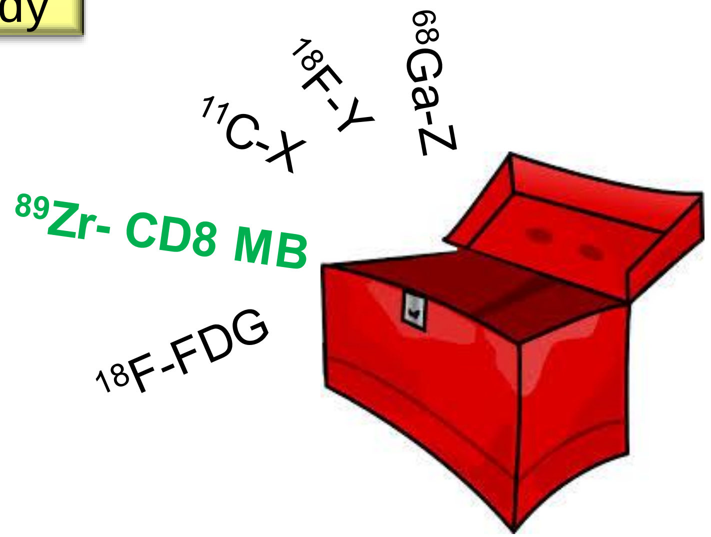
**Stage 1.** Radiolabeling / Imaging Qualifications/ Confirmations – **Preclinical PET**

→

**Stage 2.** Project Specific Questions and / or animal model Development / **Pilot Clinical PET** Study

→ →

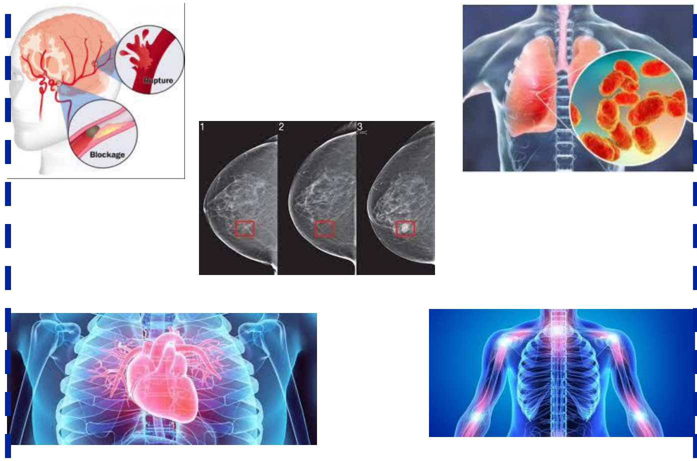
**Stage 3.** Data Driven PET **Toolbox** for clinical applications



# Summary

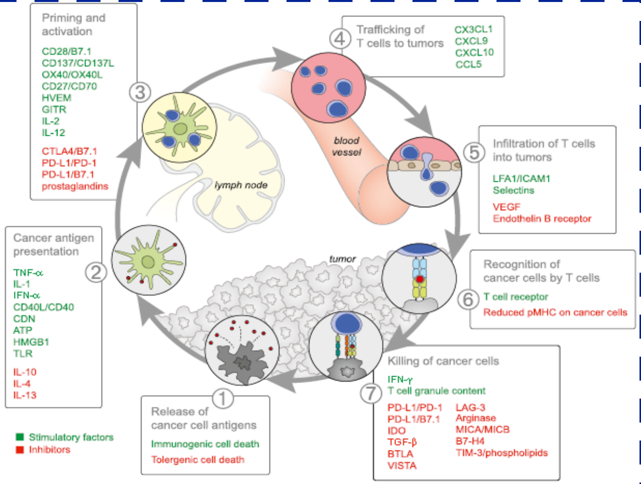
- For Pfizer, PET Imaging is a stagged approach
- Initial preclinical PET Imaging evaluates the possibilities
  - Answers questions around the “Three Pillars”
  - Allows one to evaluate dose, timing, magnitude of response
  - Overall boosts the CIR for Teams moving forward
- Pfizer designs the PET imaging clinical trials based on the preclinical data
- Clinical Application of PET imaging is a potential decision-making tool in drug development
  - Saving Time and Money

# Future Directions




❖"Every Biopsy is a failed opportunity for PET/MI"

❖How specific can we get?




❖DSTI for a reason – Next Pillar – Quantitate the Patient's well being



**Quantification of: Steps, Sedentary Time, and Moderate to Vigorous Physical Activity**

# Thanks for your attention!

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All procedures performed on animals at Pfizer were in accordance with regulations and established guidelines and were reviewed and approved by Pfizer's Institutional Animal Care and Use Committee