

Supplemental Table 1. Imaging agents in current immunotherapy trials and preclinical studies. (modified from Juergens et al, (32))

CLINICAL AGENT	CANCER	IMMUNOTHERAPY (IT) AND IMAGING TARGET	IMAGING TECHNOLOGY/ National Clinical Trials Number
¹⁸ F-FDG (52, 53)	Melanoma, renal cell, lung	IT: anti-CTLA-4, anti-PD-1 Target: tumor metabolism	PET/CT NCT01666353
¹⁸ F-FDG	Cervical, squamous cell	IT: anti-CTLA-4 Target: tumor metabolism	PET/CT NCT01711515
¹⁸ F-FDG	Multiple Cancers	IT: CAR-T, anti-CTLA-4, IL-2 Target: tumor metabolism	PET NCT02070406
¹⁸ F-FDG	Renal cell	IT: IP-2 (plus chemo) Target: tumor metabolism	PET/CT NCT01038778
¹⁸ F-FDG	Multiple Cancers	IT: CAR-T, IL-2, DC vaccine Target: tumor metabolism	PET/CT PET/MRI NCT01697527
¹⁸ F-FDG or Na ¹⁸ F	Prostate	IT: DC vaccine with GM-CSF Target: tumor metabolism	PET/CT PET/MRI NCT02042053
¹⁸ F-FET	Brain melanoma metastases	IT: anti-PD-1, anti-CTLA-4 Target: tumor metabolism	PET/MRI NCT02374242
¹¹ C-PBR28a	Brain	IT: various IT treatments Target: tumor benzodiazepine receptor	PET NCT02431572
¹⁸ F-HBG (54)	Glioma	IT: CAR-T, IL-2 Target: CAR-T cells	PET NCT01082926
⁸⁹ Zr-atezolizumab(34)	Multiple cancers	IT: anti-PD-L1 Target: PD-L1 on tumor or other cells	PET NCT02453984
⁹⁹ Tc-IL-2 (55)	Melanoma	IT: anti-CTLA-4, PD-1, IL-2 Target: TIL expressing IL-2 receptor	SPECT NCT01789827
¹⁸ F-L-FAC (56)	Healthy volunteers and multiple cancers	IT: various immunotherapies Target: activated T-cells in tumor	PET NCT01180868 NCT01180907

⁸⁹ Zr-GC1008 (57)	Brain glioma	IT: anti-TGF- β Target: TGF- β	PET NCT01472731
Ferumoxytol (nanoparticles) (58)	Brain	IT: various immunotherapies Target: macrophage in tumors	MRI NCT02452216
¹⁸ F-F-AraG (49)	Healthy subjects	IT: prior to various cancer IT trials Target: activated T-cells	PET NCT02323893
⁸⁹ Zr-Pembrolizumab	Melanoma and Non-small cell lung	IT: pembrolizumab Target: anti-PD-1	PET NCT02760225
fluorescent-bevacizumab-1RDye800CW (41)	Breast	Target: VEGFA	Optical imaging, fSTREAM NCT01508572
PRECLINICAL AGENT		TARGETING CONCEPT	IMAGING TECHNOLOGY
¹⁸ F or ⁶⁴ Cu anti-CD11b or MHC II (59)		Labeled antibody fragments binding to CD11b or MHC II on tumor macrophage or myeloid cells	PET
⁶⁴ Cu-anti-CD8 (60)		Labeled antibody fragments binding to CD8 on tumor infiltrating cytotoxic T lymphocytes	PET
⁸⁹ Zr-anti-CD8 (44)		Labeled antibody fragments binding to CD8 on tumor infiltrating cytotoxic T lymphocytes	PET
¹⁸ F-FEAU (61)		Labeled ligand identifies viral transgene in activated CAR-T that are present in tumor	PET
¹¹¹ I-anti-PD-L1 (38)		Labeled monoclonal antibody binds to PD-L1 expressed on macrophage and tumor cells	SPECT
⁸⁹ Zr-anti-CD47 (62)		Labeled monoclonal antibody binds to CD47 expressed on cells within tumor	PET
⁶⁴ Cu-Anti-CTLA-4 (63)		Labeled monoclonal antibody binds to CTLA-4 expressed on cytotoxic T lymphocytes within tumor	PET
MB-anti-B7-H3 (64)		Ultrasound microbubbles labeled with monoclonal antibody against B7H3. Identifies cells expressing B7-H3 on macrophage and tumor cells	US

⁶⁴ Cu-SPION (65)		CAR-T cells loaded with ⁶⁴ Cu-CPION (iron nanoparticles). Image accumulation of therapeutic CAR-T	PET
DiR labeled T cells (66)		DiR fluorophore, activated by near-Infrared light, is used to label T cells. T cells that located in tumor are imaged	Fluorescence imaging
⁸⁹ Zr-bispecific T cell engaging antibody (40)		IT: AMG 110 Target: epithelial cell adhesion molecule	PET

Abbreviations: CAR-T, chimeric antigen receptor T-cell; CD8, cluster of differentiation 8; CD11b, integrin alpha M; CD47, integrin-associated protein; CT, computerized tomography; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; DiR, 1,1'-dioctabecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide; ¹⁸F-FDG, fluorodeoxyglucose; F-AraG, fluoro-9-β-D-arabinofuranosylguanine; FEAU, 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-ethyluridine; FET, fluoro-ethyl-tyrosine; GC1008, fresolimumab; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBG, hydroxymethyl-butyl guanine; IL-2, interleukin-2; IT, immunotherapy; L-FAC, 1-L-(2 deoxy-2,-¹⁸Fluoroarabinofuranosyl) cytosine; MRI, magnetic resonance imaging; MB, minibody; MHC II, major histocompatibility complex 2; NCT, National Clinical Trials; PD-1, programmed cell death 1; PD-L1, programmed cell death-ligand 1; PET, positron emission tomography; PBR28, peripheral benzodiazepine receptor 28a; SPECT, single-photon emission computed tomography; SPION, super paramagnetic iron oxide nanoparticles; TGF-β, transforming growth factor-beta; TIL, tumor-infiltrating lymphocyte; US, ultrasound..

Supplemental Table 2. Molecular imaging approaches for imaging immune cells and immune responses.

	Pros	Cons	Current and future clinical use
Metabolic probes	Detect metabolically active, proliferating cells	Relatively non-specific	¹⁸ F-FDG ¹⁸ F-FLT and others
Pre-labeled cells	Low background	Need to remove, modify, reinfuse cells Dilution after cells replicate	In-111 oxine
Reporter genes	Potentially low background Can follow cells as they expand Cell surface tags	Need to modify cells (in situ or ex vivo) Potential immunogenicity of reporter genes	Non-immunogenic reporter genes (Nal symporter, huTk, etc.)
Probes for cell surface markers (antibodies, etc)	High specificity	Endogenous antigen sink Surface markers only	Human/humanized probes

Supplemental Table 3: National Institutes of Health Funding and Support

1) [General grant funding](#) includes unsolicited grants, requests for applications and specialized program announcements.

- Early Phase Clinical Trials in Imaging and IGI (R01): [PAR-14-166](#)
 - Supports early phase clinical trials
 - R01 - \$500,000 total direct costs over 2 years
- Image-guided Drug Delivery in Cancer (R01): [PAR-13-185](#)
 - Encourages innovative translational research in image-guided drug delivery (IGDD) in cancer.
- Oncology co-clinical QI imaging research resources (U24) [PAR 15-266](#)
- Academic-Industrial Partnerships for Translation of in vivo Imaging Systems for Cancer Investigations (R01): [PAR-13-169](#)
- Quantitative Imaging for Evaluation of Responses to Cancer Therapies: [PAR 14-116](#)
- NCI Informatics (U01, R01, P01, U24): [PAR 12-286-290](#)

2) Funding U.S. based small businesses

The Small Business Innovation Research (SBIR) [PA-14-071](#)

Small Business Technology Transfer (STTR) [PA-14-072](#)

3) NCI Experimental Therapeutics ([NExT](#)) program (67)

Provides direct access to NCI resources and expertise, but it is not a grant. NCI performs approved portions of the project for the applicant using its internal resources.

Resources that NCI can provide are:

- Multi- and interdisciplinary clinical/translational teams
- Early access to leading-edge translational technologies
- PK/PD modeling and assay development
- Toxicology/Safety Pharmacology
- Formulation & GMP Scale-Up
- Imaging for biodistribution
- Development & validation of pharmacodynamics assays
- Development & validation of clinical assays
- Proof-of-concept or first in human studies
- <https://next.cancer.gov/experimentalTherapeutics/form.htm>

ABBREVIATIONS

CT	computed tomography
CAR-T	chimeric antigen receptor T cells
¹⁸ F-FDG	fluorodeoxyglucose
FLT	fluorothymidine
HER2	human epidermal growth factor receptor 2
IDO	indoleamine 2,3 dioxygenase
MATCH	Molecular Analysis for Therapeutic Choice Trial
MRI	magnetic resonance imaging
NCI	National Cancer Institute
NEXT	NCI Experimental Therapeutics Program
NIH	National Institutes of Health
PET	positron emission tomography
RECIST	response evaluation criteria in solid tumors
TME	tumor microenvironment

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