Cancer Associated Fibroblast (CAF) and current Clinical trial Studies of Diagnostic Agents

By

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I have no financial disclosures to claim

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Objectives

1. Describe the Tumor Micro Environment (TME), its formation, and components along with Fibroblast Activated Protein (FAP(I) and its role in tumors
2. Discuss multiple cancer type detection using Fibroblast labeled with a radiotracer and role of CAF
3. Analyze some clinical trials/studies using fibroblast PET tracers for detection

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Assessment question1

The Tumor Micro Environment (TME) can be described as:

a) A simple cell
b) A non evolving cell
c) A cell that can passively promote cancer progression
d) A cell that can perform pro and anti tumorigenic function

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Assessment question 2

The TME component that plays a critical step in the tumor formation is:

a) Dendritic cells
b) Macrophages
c) Stromal cells
d) T-cells

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Assessment question 3

Of the following product, which was used to prevent radiolysis in the F-18 FAPI74 one-pot method?

a. Phosphate buffer
b. Ascorbic acid
c. Sodium acetate
d. Sodium ascorbate

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Assessment question 4

PDCs can be described as:

a. Non-specific release of protease
b. Complex protein
c. Linked protein
d. Intracellular warhead

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Assessment question 5

ADCs can be described as:

a. Tumor specific linked protein
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c. Antibody drug conjugate
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Assessment question 6

The purpose of hygroscopic DMSO in the preparation of FAPI-46 is:

a. As an antioxidant
b. As a solubilizer
c. As a ligand
d. As a chelator

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Objective 1

The Tumor Micro Environment (TME) and Fibroblast Associated Protein (FAP)

Describe the Tumor Micro Environment (TME), its formation, and components along with Fibroblast Activated Protein (FAP(I) and its role in tumors

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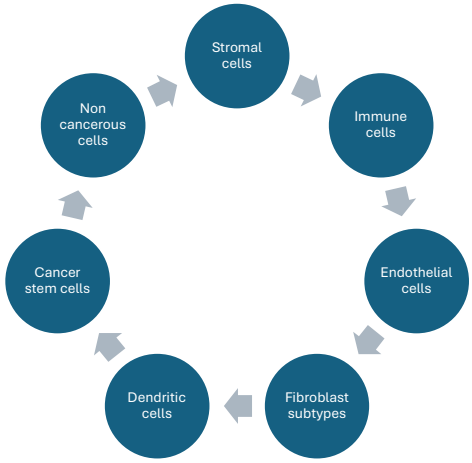
Complexity of the Tumor Cell



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Complexity of the tumor cell (continued)

Complexity of the tumor cell is based on understanding the TME (Tumor Micro-Environment)

- Stromal cell
- Cell-cell interaction
- Cell-matrix interaction
- Abnormal cell physiology

TME is the environment around the tumor

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Stromal Cells

Within the stromal cells are:

- Endothelial: can affect glycolytic genes, phosphorylation, and several markers
- Immune: includes macrophages B-Cells and T-cells
- Fibroblast subtypes
- Cancer Associated Fibroblast
- Several other cells
- Cell-cell interaction
- Critical step and component in the formation of the tumor micro-environment

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The Extracellular Matrix - ECM

Complex network of:

- proteins
- polysaccharides
- Growth factor

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The Tumor Micro-Environment (TME)

- Tumor Micro-Environment
- Composed of various cells: immune, endothelial cells, blood vessel, and fibroblast
- Is complex
- Is continuously evolving through interaction with the tumor and its surrounding
- Can actively promote cancer progression
- Can coordinate a program that promotes angiogenesis
- Can perform both pro and anti-tumorigenic functions
- Can be both cancerous and non- cancerous

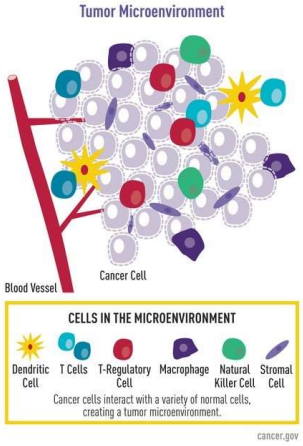

Photo: courtesy of National Cancer Institute

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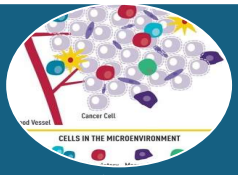
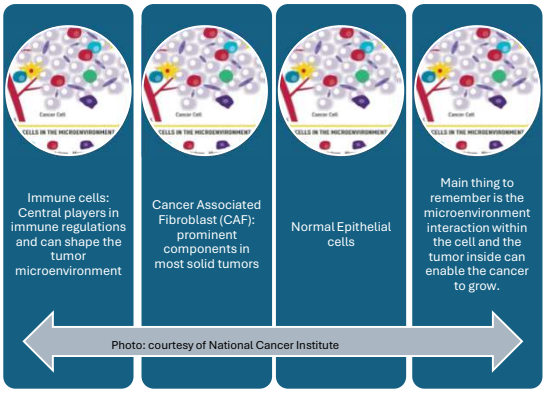
Formation of the TME



Formation of TME
Can form from diverse types of cells

Photo: courtesy of National Cancer Institute



Immune cells: Central players in immune regulations and can shape the tumor microenvironment

Cancer Associated Fibroblast (CAF): prominent components in most solid tumors

Normal Epithelial cells

Main thing to remember is the microenvironment interaction within the cell and the tumor inside can enable the cancer to grow.

Photo: courtesy of National Cancer Institute

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Tumor Microenvironment (TME) (continued)

- Immune cells –central players
- Endothelial cells – may or may not involve the lymph nodes
- Can contain fibroblast – prominent in solid tumors
- Pericytes
- Fibroblasts
- Stromal cells
- Blood vessels
- Non-cancerous components comprising TME is central to all stages of tumorigenesis, metastasis, and tumor progression

Immune cells: contain T-lymphocytes and B-lymphocytes T-lymphocytes: confer cellular immunity – can influence tumorigenesis

T- cells - Detect abnormal tumor antigens for destruction

B-Lymphocytes: humoral immunity – found in lymph nodes and close proximity to the TME - important during tumorigenesis

Immune cells: critical component of the TME – can either

suppress tumor growth

Promote tumor growth

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TME components of mention

- TME components that contributes to all aspects of tumor progression includes:
- Myeloid cells with tumor associated macrophages
- Neutrophils: 1st line of defense against many pathogens. Hence can either suppress or promote tumor growth
- Basophils
- Vary significantly between tumor types
  Include vascular endothelial cells, fibroblasts, adipocytes and stellate cells
- Dendritic Cells: critical role is in the immune system
  They can recognize, capture, and present antigens to the T-Cells (mainly in the lymph nodes)
- Stromal Cells: critical steps in tumor formation. These stromal cells can alter the extracellular matrix during carcinogenesis

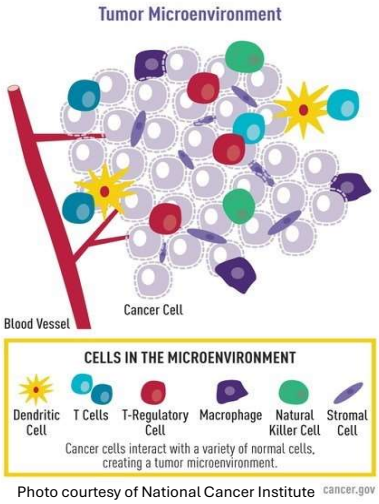

Photo courtesy of National Cancer Institute cancer.gov

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Cancer Associated Fibroblast (CAF)

- Also known as activated fibroblast
- Located in the center of the stroma within the TME
- Prominent components in most types of solid tumors
- Facilitate cancer progression by:
- Supporting tumor cell growth
- Involvement in the extracellular matrix remodeling
- Promote angiogenesis
- Mediate tumor-promoting inflammation

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CAF Derivation

- Fibroblast
- Epithelial cells
- Endothelial cells
- Cancer stem cells
- Adipocytes
- Pericytes stellate cells
- Pericytes: silent resident fibroblasts in the liver or pancreas; can acquire a CAF phenotype upon activation by Tumor Growth Beta Factor (TGBF) and Platelet Derived Growth Factor (PDGF)

CAF are a combination of all these cells

- Maintain a role in various function of the tumor
- Can contribute to immune regulation, angiogenesis, and ECM remodeling
- Contribute to tumor promoting inflammatory process and fibrosis
- Are usually associated with cancer

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Objective 2

Discuss multiple cancer type detection using Fibroblast labeled with a radiotracer and role of CAF

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Epithelial Carcinomas

CAF present in 90% of epithelial carcinomas and have an increase number of stromal cells.

epithelial carcinomas include:

- Pancreatic
- Colon
- breast cancers
- Liver
- Lung
- Esophageal

Tumors with increase stromal cells: worse prognosis, treatment resistance, and disease relapse.

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Role of CAF

- Central players in immune regulation
- CAF shapes the microenvironment (TME) of the tumor
- Exhibit several pro-tumorigenic functions
- CAF also contribute to the immune escape mechanism by upregulating the immunosuppression of cytokine production and immune checkpoint ligands
- CAF have increase expression of Fibroblast Activation Protein (FAP)
- Are major component of the tumor stroma

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Fibroblast Activated Protein (FAP)

Vital in two specific areas

- Tumor invasion
- metastasis

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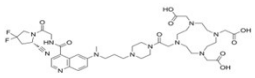

FAPI-46

- Can be produce from Ge68/Ga68 generator
- Quinoline-based FAP targeted radiotracer
  DOTA ligand molecule
- Has shown to have high tumor uptake and prolonged accumulation in the tumor
- Hygroscopic DMSO – significantly impact its solubility

currently involved in several different clinical trials – involving the following conditions: Metastatic malignant neoplasm in the prostate gland/prostate carcinoma (phase 1)

Breast carcinoma/Colon carcinoma/esophageal carcinoma/gastric carcinoma (Early phase 1)

Bladder carcinoma/cervical carcinoma/hematopoietic and lymphoid cell neoplasm/hepatocellular carcinoma (phase 1)

Pancreatic cancer (Phase 2) NCT05262855

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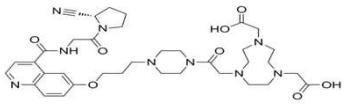
FAPI74



- PET tracer that targets FAP
- Can be used to image FAP(+) tumor
- Synthesized with F-18
- Is a NOTA ligand molecule
- Cyclotron produced
- Can only be prepared off site unless the facility possess a cyclotron

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Comparison of FAPI-46 and FAPI74

Ga-68 FAPI-46	F-18 FAPI74
Generator produced	Cyclotron produced
Short t1/2 = 68min	Longer t1/2 = 110min
Can be prepared on site	Cannot be prepared on site
Positron emitter	Positron emitter
Must perform weekly Ge68 breakthrough test	Weekly test not necessary

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Objective 3

Analyze current on-going clinical trials/studies using fibroblast activated protein

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NIH study F-18-FAPI74

Study objective: to determine if F18-FAPI74 is as good or better than current available methods for detecting certain cancers

Inclusion criteria:

Patients must be ≥18 y.o.

Cancers included in the study: ducts of the pancreas, liver, gallbladder or small intestine, stomach, bladder, ovaries, adrenal glands

Exclusion criteria

Allergic reaction to any of the tracers including F-18 FAPI74

History of severe claustrophobia

Body weight >350 lbs

(+) Beta-HCG test

Scr>2x upper limit

Liver transaminase >3x upper limit

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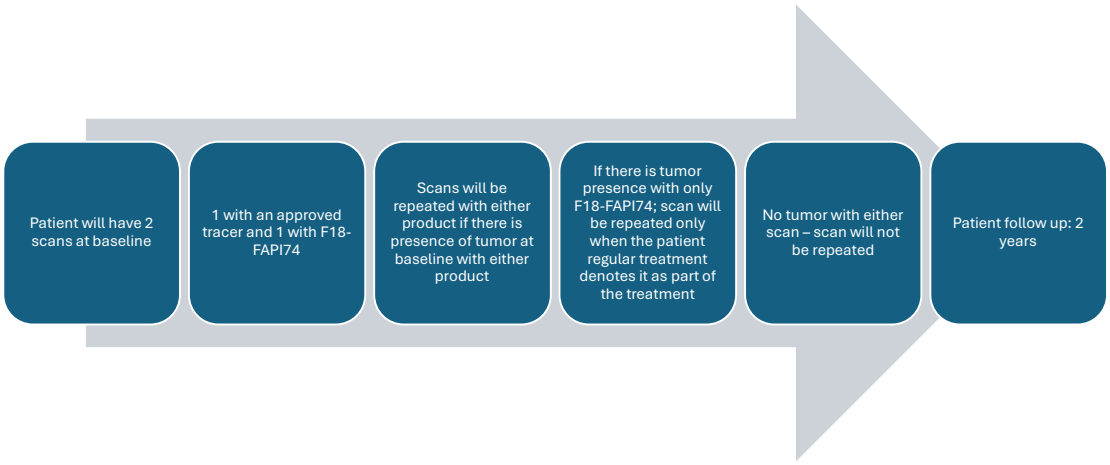
NIH Study design



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NIH Study type and phase

Phase II study (still ongoing)	Study type: diagnostic	Trial ID: 10001731, NCI-2024-06149, 001731-C	Clinicaltrials.gov ID: NCT06503146

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The following study : "A tumor-Imaging method targeting Cancer-Associated Fibroblasts"

Also an NIH study

- Methods: utilized an iodinated and a DOTA-FAPI specific products
- Involvement: In vitro uptake, competition and efflux studies, microscopic fluorescence-labeled variant and
- Biodistribution of FAPI-Ga68 labeling – high contrast imaging, negligible exposure to radiation for the healthy tissues, fast body clearance
- When compared with F-18FDG scan in patient with advanced lung carcinoma, the DOTA FAPI ligand was superior
- Study Conclusion: Radiolabeled FAPI allows for faster imaging with high contrast in tumors with high stromal content
- DOTA or other chelators offers the labeling not only with Ga68 but also to other therapeutic isotopes such as Lu-177 or Y-90.

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Meta-analysis of FAP Overexpression

- Objective: to clarify prognostic value in different tumors
- The global analysis that was performed through PubMed, EMBASE, and NCKI included 15 studies with various solid tumors
- In this global analysis: FAP overexpression had significant associations with poor OS and tumor invasion
- OS: HR=2.18, P=0.007
- Lymph node metastasis: OR=3.80, P=0.004
- Meta-analysis concluded that FAP overexpression has worse prognosis in solid tumors especially when found in the tumor cells rather than the stroma cells.

Disease state included in the meta-analysis

- Epithelial ovarian carcinoma
- Pancreatic adenocarcinoma
- Osteosarcoma
- Esophageal adenocarcinoma
- Colorectal cancer
- Gastric cancer
- Endometrial carcinoma
- Medullary thyroid carcinomas
- Invasive ductal carcinoma of the breast
- Oral squamous cell carcinoma

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Clinical study by Watabe et al.

- Conducted in Japan at Osaka University
- Total of 31 patients with various cancers included
- Cancers had to be confirmed via histopathology
- Various types of cancers: lung cancer, squamous cell carcinoma, gastric, pancreatic, oropharyngeal, thymic and thymoma and benign tumors
- 18FAPI74 compared to F-18FDG scan

- Results: F-18 FAPI74 showed higher uptake in primary lesions than F-18 FDG in malignant lesions
- Some of the non malignant lesions also showed high uptake
- F-18 FAPI74 also showed significant higher uptake than F-18 FDG in lymph node metastases

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Osaka University Results

		Osaka University Results			
		Median SUV max			
Cancer types		F-18 FAPI74	F-18 FDG		P value
1° lesions of various cancer types		9.39 [range, 1.83-25.28]	3.49 [range, 2.21-15.58]		0.0053
1° lesions		9.44 [range, 2.50-25.28]	5.45 [range, 1.22-15.06]		0.010
lymph node metastases		8.86 [range, 3.51-23.33]	3.84 [range, 1.01-9.75]		0.002
other metastases		6.39 [range, 0.55-12.78]	1.88 [range, 0.7-8.35]		0.046

Table by G. Simon-Clarke

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Another Japan study (F18-FAPI74 one-pot method)

			F-18FAPI-74 One-Pot-Method (n=10)						
			Multi-purpose synthesizer (CFN-MPS 200)						
Diluent type	# of samples	RCP @EOS T_0	RCP after 2hrs T_2	RCP after 3hrs T_3	RCP after 4 hrs T_4	Synthesis time	radiochemical yield	Radioactivity @EOS	
Saline #1 (n=1)	1	98%	96%	79%	54%	31 min	37%	32Gbq - 40Gbq	
Saline #2 (n=1)	1	97%	96%	94%	89%	31 min	37%	32Gbq - 40Gbq	
Saline +10mg Ascorbic acid +90mg NaAscorbate (pH 5.0) (n=1)	1	98%	97%	96%	95%	31 min	37%	32Gbq - 40Gbq	
Saline +100mg NaAscorbate (n=1)	1	98%	97%	97%	96%	31 min	37%	32Gbq - 40Gbq	
phosphate buffered saline (pH 7.4) (n=1)	1	98%	96%	96%	95%	31 min	37%	32Gbq - 40Gbq	
10mM phosphate buffered saline (pH 6.7) (n=1)	1	98%	96%	95%	89%	31 min	37%	32Gbq - 40Gbq	
phosphate buffered saline (pH 7.4)+100mg NaAscorbate (n=1)	1	98%	97%	97%	97%	31 min	37%	32Gbq - 40Gbq	
10mM phosphate buffered saline (pH 6.7)+100mg NaAscorbate (n=3)	3	98%	97%	97%	97%	31 min	37%	32Gbq - 40Gbq	

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F-18 FAPI-74 One-Pot-Method Result

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Avacta Phase 1 trial

Goal : to develop theragnostic agent that target FAP (Fibroblast Activated Protein)

Platform for the drug is Precision(TM) drug conjugate (PDC), not ADC (Antibody Drug Conjugate)

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Avacta Clinical trial

This trial is taking place in the US

Inclusion criteria: patient with FAP diagnosed tumors

Exclusion criteria: none listed

Type of study: open label

Phase 1 study

Agent used: All in one mini-cassete-FAPI74

Synthesis is ~ 20 minutes

Result: on going

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Peptides and Antibodies

Peptides	Antibodies
Small molecules mwt ≤ 10kDa	Large molecule (mwt 50 kDa to 150kDa)
Susceptible to enzymatic degradation by plasma protease and peptidase	Slow blood clearance
Rapid blood clearance due to their small molecular weight	Less susceptible to enzymatic degradation
Less immunogenic	Intracellular membrane accessible
Readily accessible on extracellular membrane	Less effective in solid tumors – lack of access to tumor cells
	More likely to be immunogenic

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PDC vs ADC

PDC (Protein Drug Conjugate)	ADC (Antibody Drug Conjugate)
Linked protein that is tumor specific	Non-specific release of the protease which can cause lung toxicity
1:1 drug peptide	4.8:1 drug to antibody
MOA: extracellular warhead release that is more on target causing less systemic exposure	MOA: intracellular release of the ADC. It is a complex process and is necessary for the action to occur

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Study of F-18 FAPI74 in patients with Intestinal Cancers

- Prospective, multi-center, Open Label study
- Study type: Diagnostic, interventional
- Phase 2, ongoing study
- Non randomized
- Inclusion criteria: pts with hepatocellular carcinoma, cholangiocarcinoma, gastric, pancreatic, and colorectal cancer
- Goal of study: to assess efficacy of F-18 FAPI74 in detection of above cancers
- Methods: PET scan results vs FAP immunohistochemistry (1º objective) and histopathology (2º objective) of biopsied or tissue resection.

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Any questions?

Thank you

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