Antibody Blockade of Semaphorin 4D Sensitizes Pancreatic Cancer to Immune Checkpoint Blockade

Disclosures

• I have no conflicts of interest to disclose
Rising Burden of Pancreatic Cancer


Conroy et al. NEJM 2011
Rising Burden of Pancreatic Cancer

5-Year Survival By SEER Staging

Source: SEER Cancer Statistics
PDAC Tumor Microenvironment Thwarts Adaptive T-Cell Response

<table>
<thead>
<tr>
<th>H &amp; E</th>
<th>Trichrome</th>
<th>Sirius Red</th>
<th>αSMA</th>
<th>CD 45</th>
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<tbody>
<tr>
<td>Normal Pancreas</td>
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<tr>
<td>Primary PDAC</td>
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<tr>
<td>Metastatic PDAC</td>
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PDAC Tumors Are Immunologically Cold and Unresponsive to Immune Checkpoint Blockade


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Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma.
Royal RE\textsuperscript{1}, Levy C, Turner K, Mathur A, Hynie M, Kammula US, Sherry RM, Topalian SL, Yang JC, Lowy I, Rosenberg SA

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Durvalumab With or Without Tremelimunab for Patients With Metastatic Pancreatic Ductal Adenocarcinoma: A Phase 2 Randomized Clinical Trial.
C Relli EM\textsuperscript{1}, QH DY\textsuperscript{2}, Dhar H\textsuperscript{3}, Reinaud P\textsuperscript{4}, Lee MA\textsuperscript{5}, Sun W\textsuperscript{6}, Fisher G\textsuperscript{7}, Honzak A\textsuperscript{8}, Chang SC\textsuperscript{9}, Vanovic G\textsuperscript{10}, Takahashi G\textsuperscript{11}, Yang Y\textsuperscript{12}, Fitts D\textsuperscript{13}, Philip PA\textsuperscript{14}

0% ORR in patients with PDAC

0% ORR

3% ORR
Semaphorin-Plexin Signaling Drives Suppression of T-Cell Response in Murine Models of Solid Tumors

Peacock et. al. EMBO Mol Med., 2018

Clavijo et. al. CIR, 2019
Evans et. al. CIR, 2015
Semaphorin 4D is Overexpressed in the TME of Human PDAC

n=11 per group, p<0.001
Human PDAC

Leukocytes

CD 55
11.8

Myeloids

CD 11b
51.7

TAMs

40% of CD 45

CD68

TAMs 80.5%
Plexin B2+

MHCII

Lymphocytes

86.5% SEMA 4D+

SEMA4D

CD4
56.0%

CD8
31.6%

CD3
30.7%

Plexin B2

40% of CD 45

Human PDAC

Leukocytes

CD 45
15.9%

Lymphocytes

CD3
86.5%

CD8
30.7%
SEMA4D and Its Receptor Plexin B2 Are Co-expressed by TAMS
Orthotopic Murine Model of PDAC Closely Recapitulates Human PDAC

Orthotopic KP2 PDAC

Human PDAC

C57b/6 p48-Cre;
KRASG12D; TP53 -/WT

High Frequency Ultrasonography

Orthotopic Murine Model of PDAC Closely Recapitulates Human PDAC
Leukocytes

Myeloids

Monocytes

Granulocytes

Ly6C

Ly6G

F4/80

TAMs 80% Plexin B2+

MHCII

CD 8, 94% SEMA4D+

CD 45

CD 11b

Ly6 G

Plexin B2

CD 3

CD 4

FSC-A

AKAT SeminID, FSC-A subset 94.3%
SEMA4D Blockade Augments Dual Checkpoint Therapy in The Context of FOLFIRINOX

* Log-Rank < 0.05 compared to all other groups, n=20 mice per group. FOLFIRINOX Dosed Weekly, Immunotherapy BIW
SEMA4D Blockade Reduces PDAC Tumor Burden

Tumor Weights At Sacrifice

- $\alpha$PD+ $\alpha$CTLA4+ $\alpha$SEMA4D
- $\alpha$PD+ $\alpha$CTLA4+ Isotype Control
- Isotype Controls + $\alpha$SEMA4d
- Isotype Controls

Multiparameter Flow Cytometry

![Graph showing tumor mass comparisons](graph.png)
SEMA4D Blockade Increases Tumor Penetration of CD8 T-Cells

**Vehicle**

**ICB**

**αSEMA**

**SEMA + ICB**

Tumor Infiltrating CD8 T Cells

CD8 T-Cells Per Gram Tissue


g-SEMA4D + ICB

ICB + Isotype

CD8 T-Cells

CD4 T-Cells

**Figure:**

- **Left Panel:** ICB + Isotype
  - CD4: 37.6%
  - CD8: 18.8%

- **Center Panel:** αSEMA4D + ICB
  - CD4: 33.1%
  - CD8: 56.1%

- **Right Panel:** Tumor Infiltrating CD8 T Cells
  - Comparison of Vehicle, ICB, αSEMA, αSEMA + ICB
  - Statistical significance marked with * and **.
SEMA 4D Blockade Abrogated SEMA4D Signal Within TME

- Untreated CD3+, SEMA4D
- Treated CD3+, SEMA4D
- SEMA 4D FMO
- Treated CD3+, SEMA4D
SEMA4D Blockade Turns Immune “Cold” Tumors “Hot”

Tumor Infiltrating Immune Cells

- Vehicle
- ICB
- αSEMA
- αSEMA + ICB

CD8 Tumor Infiltration

- Vehicle
- ICB
- αSEMA
- αSEMA + ICB

Macrophage Infiltration

- Vehicle
- ICB
- αSEMA4D
- αSEMA4D + ICB
SEMA4D Blockade Shifts Innate Immunity Towards Antigen Presentation

**MHC Class II Expression by Macrophages**

- Vehicle
- ICB
- αSEMA4D
- αSEMA4D+ICB

Mean Fluorescent Intensity

- **20,000**
- **30,000**
- **40,000**
- **50,000**
- **60,000**
- **70,000**

**M2 TAM Tumor Infiltration**

- CD206hi / F4/80 Macrophages

- Vehicle
- ICB
- αSEMA4D
- αSEMA4D+ICB

Mean Fluorescent Intensity

- **0.0**
- **0.1**
- **0.2**
- **0.3**
- **0.4**

*Significant differences indicated by asterisks.*
Phase 1b

- BOIN Enrollment Rules (18-66 patients)
- Dose de-escalation
- Folfirinox + anti-PD1/PD-L1 + VX15/2503

Phase 2
Expansion Cohort

- Simon’s Two Stage Design Interim Assessment (18-46 patients)
- MTD Dose of Folfirinox + anti-PD1/PD-L1 + VX15/2503

Recruitment by Medical Oncology
Assessment of eligibility
Consent

Baseline physical exam, Baseline biopsy, Peripheral blood draw. Enroll into Protocol

After 2nd cycle, Obtain on-treatment biopsy, Peripheral blood Draw

After completion of 12th cycle, continue on immunotherapy at discretion of treating physician
Collect up to five 18 Gauge Needle Biopsies of Tumor Tissue By Interventional Radiology or Gastroenterology

**Single-Cell/Bulk Genomics**

1 Core Needle Biopsy for bulk and tissue permitting, Single Single-Cell RNA-Seq. Population Subtyping of Tumor, Normal Tissue and Stromal Compartments

**Immune Mass-Cytometry**

1-2 Core Needle Biopsies Digested Into Single Cell-Suspension, 1 Million Cells Stained with T-Cell and M-Cell Mass Cytometry Panels And Analyzed For Phenotypic and Functional Immune Markers

**Quantitative Stromal IHC**

1 Core Needle Biopsy Formalin Fixed and Paraffin Embedded For Sectioning and Staining for Stromal Elements Including Collagen, Vasculature, and Fibroblast Markers. Quantified on Aperio Versa System. GENIE Machine Learning Driven Analysis and Correlation

**Multi-Dimensional Analysis Incorporating Transcriptomic, Proteomic, and Tumor Immune Microenvironment Composition To Predict Response to Treatment and Mechanism of Treatment Resistance**
Phase 1b: Enrollment begins at dose 0, and proceeds by BION rules dependent on observed rates of DLT, target DLT rate 0.3. Patients enroll via Bayesian Optimal Interval Design (BOIN). Model detects Maximal Tolerated Dose (MTD) with a target DLT rate of 0.3. All patients evaluated for secondary endpoints.

Phase 1b (n=28):
- Patients continue to enroll via BOIN rules until n=18 patients have been enrolled to a single dose yielding a 0.3 DLT rate.
- After 18 patients enroll and are evaluated for RECIST 1.1 at MTD, evaluation of futility based on Two Stage Design occurs in this population.

Simon’s Two Stage Rules:
- ≤6 responses: Do not expand into Phase 2 cohort given futility.
- >6 responses: Begin Phase 2 expansion cohort, with 28 additional patients at MTD (n=46 total patients).

Phase 2: Accrual continues in Phase 1b until the 18th patient is evaluated for RECIST 1.1. Phase 2 Enrollment begins once 18th evaluation passes Simon’s Two Stage Rule. Total number of patients between Phase1b/2; n= 18-94, but expected to be 56.
Thank you

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