Characterization of a novel immunohistochemistry (IHC) assay for CEACAM5 using a commercial antibody

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INTRODUCTION

- Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) is expressed on the surface of some tumor epithelial cells and is a potential therapeutic target for antibody-drug conjugates, such as tusamitamab ravtansine, in development[1-3]
- An immunohistochemistry (IHC) assay has been validated in multiple cancer indications, including non-small cell lung cancer (NSCLC), for evaluation of clinical samples using a proprietary CEACAM5-specific murine antibody (Sanofi CEACAM5 IHC monoclonal antibody clone 769 [Sanofi clone 769]) and the Dako/Agilent Autostainer Link 48 platform[4]
- Here, we compare 5 commercially available monoclonal antibody (mAb) clones targeted to CEACAM5 with the validated CEACAM5 antibody Sanofi clone 769

METHODS

- The commercially available mAb clones tested are shown in **Table 1**

Table 1. Commercially available monoclonal antibody clones evaluated

Commercial provider	Clone number	Species and Ig isotype	Target	Catalog number
Santa Cruz Biotechnology	CI-P83-1	Mouse IgG1	Human CEACAM5	sc-23928
R&D Systems	487609	Mouse IgG2a	Human CEACAM5 (amino acids 35–685)	MAB41281
Thermo Fisher	OTI1D4	Mouse IgG2b	Human CEACAM5 (amino acids 35–680)	TA803413
Abcam	EPCEAR7	Rabbit IgG	Human CEACAM5	ab133633
Sino Biological	327	Rabbit IgG	Human CEACAM5	11077-R327

Ig, immunoglobulin.

- IHC staining was optimized and evaluated in formalin-fixed, paraffin-embedded (FFPE) human cancer tissues and cell pellets from control cell lines
  - The staining pattern and intensity of CEACAM5 reactivity were compared for the commercial clones and clone 769 in the same FFPE samples
- IHC staining for the commercial antibodies used the Leica BOND III platform (Leica Biosystems, Deer Park, IL), whereas the validated Sanofi clone 769 assay used the Dako/Agilent Autostainer Link 48 platform (Agilent, Santa Clara, CA)
- Initial IHC testing used gastric cancer (GC) tissues
- To establish optimal antibody dilutions and the range and linearity of the tests, further assay optimization for the best performing initial clones used both GC and colorectal cancer (CRC) tissues
- Accuracy testing to evaluate staining accuracy and specificity for CEACAM5 using the optimized antibody concentrations that were established in earlier testing was done in cell line controls and additional human cancer tissues (NSCLC adenocarcinoma; NSCLC squamous cell carcinoma; small cell lung cancer; and gastric, pancreatic, and colorectal cancers)
- Final IHC assay conditions are shown in **Table 2**

Table 2. CEACAM5 IHC assay conditions

	Sanofi clone 769	Santa Cruz Biotechnology clone CI-P83-1
Monoclonal antibody dilution		
In indications other than CRC	0.5 µg/mL	0.75 µg/mL
In CRC	0.0083 µg/mL	0.0125 µg/mL
Incubation time	30 min	15 min
Pre-treatment	FLEX Target Retrieval Solution, Low pH	Epitope retrieval solution 2, no enzyme
IHC platform	Dako/Agilent Autostainer Link 48	Leica BOND III
Detection system	Mouse EnVision FLEX kit	BOND Polymer Refine Detection kit
Chromogen	DAB	DAB

CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; CRC, colorectal cancer; DAB, 3,3'-diaminobenzidine; Ig, immunoglobulin; IHC, immunohistochemistry.

- Fit-for-purpose validation of the Santa Cruz Biotechnology clone CI-P83-1 involved sensitivity screening/precision and reproducibility testing and scoring by a board-certified pathologist alongside the Sanofi clone 769 assay in a panel of 33 nonsquamous NSCLC tissues

Materials

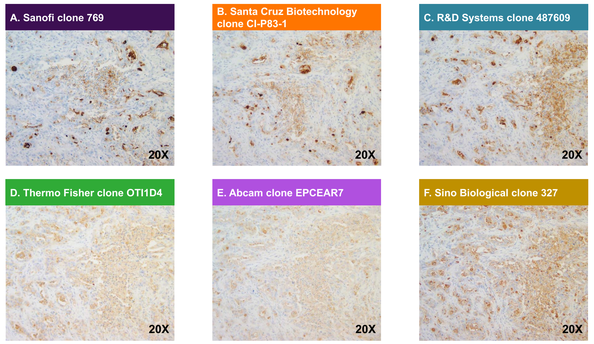
- All human cancer tissues were from Discovery Life Sciences (Newtown, PA) and were either multi-tissue blocks or single tissue
- Human cancer tissue samples were ethically obtained with written patient consent
- Cell lines for endogenous CEACAM5 expression were provided by Sanofi and included anticipated positive controls of HPAC (pancreatic adenocarcinoma), HPAF (pancreatic adenocarcinoma) and MKN-45 (gastric adenocarcinoma) cells and a negative control of HEK293T cells
- Chinese hamster ovary (CHO) cells overexpressing CEACAMs 1, 5, 6, 7, and 8 were also provided by Sanofi

RESULTS

Comparison of antibodies in GC tissues

- Of the 5 antibodies tested, 2 clones sufficiently matched the staining pattern and intensity of the reference clone (Sanofi clone 769) in GC tissues; the remaining 3 clones were deemed unsatisfactory due to diffuse cytoplasmic staining or overstaining and were not evaluated further (**Figure 1**)
  - The Santa Cruz Biotechnology CI-P83-1 and R&D Systems 487609 clones (**Panels B and C**) best matched the pattern and intensity of Sanofi clone 769 staining (**Panel A**)
  - The Thermo Fisher OTI1D4 and Abcam EPCEAR7 clones (**Panels D and E**) showed reactivity to CEACAM5 but also included higher levels of diffuse cytoplasmic staining
  - The Sino Biological clone 327 (**Panel F**) showed similar staining to that of Sanofi clone 769 but also sometimes showed overstaining

Figure 1. CEACAM5 staining in gastric cancer tissues.*



*Samples were 1 of 9 tissues in a multi-tissue block of gastric adenocarcinoma tissues.
CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5.

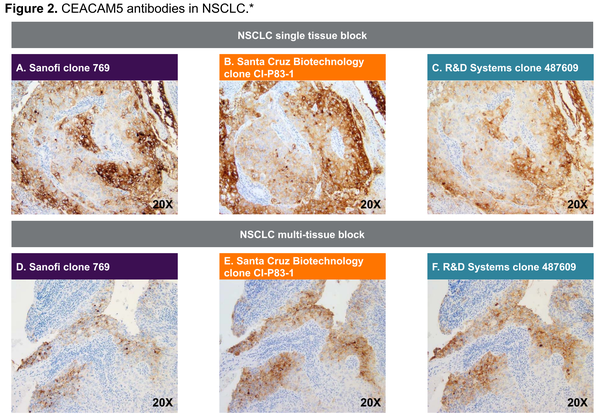
Assay optimization

- Based on the initial results in GC, the Santa Cruz Biotechnology clone CI-P83-1 and R&D Systems clone 487609 were further tested to establish optimal primary antibody dilutions that best matched the intensity of staining observed with Sanofi clone 769 and to demonstrate the range and linearity of the tests in GC and CRC
  - The validated IHC assay for Sanofi clone 769 uses a 60-fold lower antibody concentration in CRC indications compared with concentrations used for other indications
  - Optimized antibody concentrations were determined to be 0.75 µg/mL in GC (and other non-CRC solid tumors) and 0.0125 µg/mL in CRC for Santa Cruz Biotechnology clone CI-P83-1 and 0.04 µg/mL in GC (and other non-CRC solid tumors) and 0.004 µg/mL in CRC for R&D Systems clone 487609

Further comparison of antibodies in human cancer tissues, including NSCLC

- When optimized antibody dilutions were used, although both the Santa Cruz Biotechnology clone CI-P83-1 and R&D Systems clone 487609 had staining results comparable to those of Sanofi clone 769 in NSCLC tissues, parity to Sanofi clone 769 was greater for the Santa Cruz Biotechnology clone CI-P83-1 than for the R&D Systems clone 487609, which sometimes showed slightly higher CEACAM5 signal than that of the Sanofi clone 769 (**Figure 2**)
  - Similar results were observed in gastric, colorectal, pancreatic, and small cell lung cancers (data not shown)

Figure 2. CEACAM5 antibodies in NSCLC.*

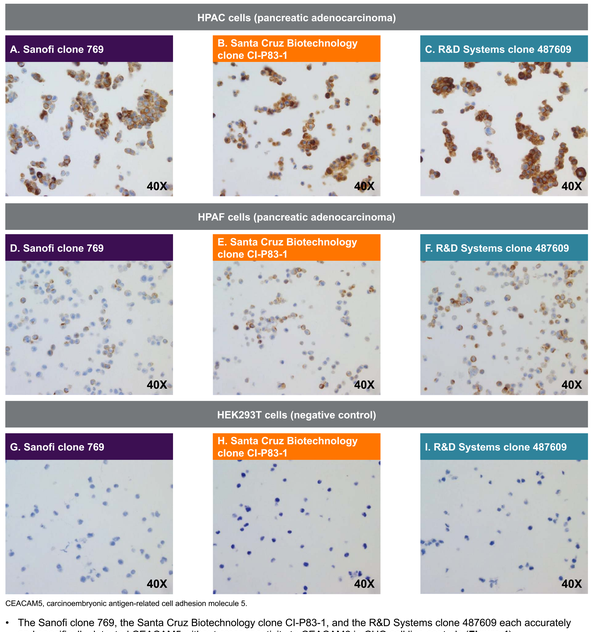


*Panels A–C are from a single tissue block of NSCLC (adenosquamous with prominent lymphoplasmacytic infiltrates). Panels D–F are from a multi-tissue block comprising 8 different NSCLC tissues (4 adenocarcinoma and 4 squamous cell carcinoma).
CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; NSCLC, non-small cell lung cancer.

Comparison of antibodies in CEACAM5 expression control cell lines

- The Santa Cruz Biotechnology and R&D Systems clones were tested at their optimized assay conditions against cell lines serving as positive or negative expression controls for endogenous CEACAM5 expression (**Figure 3**)
  - Equivalent positive staining occurred among the Sanofi clone 769 and both the Santa Cruz Biotechnology CI-P83-1 and R&D Systems 487609 commercial clones in cell lines expected to express CEACAM5: HPAC (pancreatic adenocarcinoma) and HPAF (pancreatic adenocarcinoma) (**Panels A–F**)
    - Similar staining results were observed in MKN-45 (gastric adenocarcinoma) as in the other positive controls (data not shown)
  - All three CEACAM5 clones were equally nonreactive in the HEK293T (derivative of human embryonic kidney 293 cells) negative control cell line (**Panels G–I**)

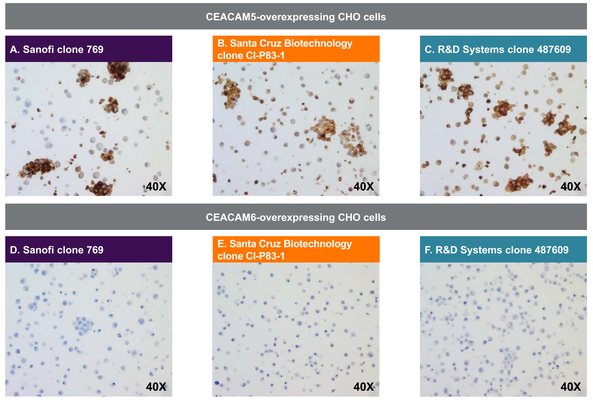
Figure 3. CEACAM5 antibodies in cell line expression controls.



CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5.

- The Sanofi clone 769, the Santa Cruz Biotechnology clone CI-P83-1, and the R&D Systems clone 487609 each accurately and specifically detected CEACAM5 without cross reactivity to CEACAM6 in CHO cell line controls (**Figure 4**)
  - Additionally, the three antibodies tested did not demonstrate cross-reactivity with CHO cell line controls overexpressing CEACAM types 1, 7, and 8 (data not shown)

Figure 4. CEACAM5 antibodies in cross-reactivity cell line controls.

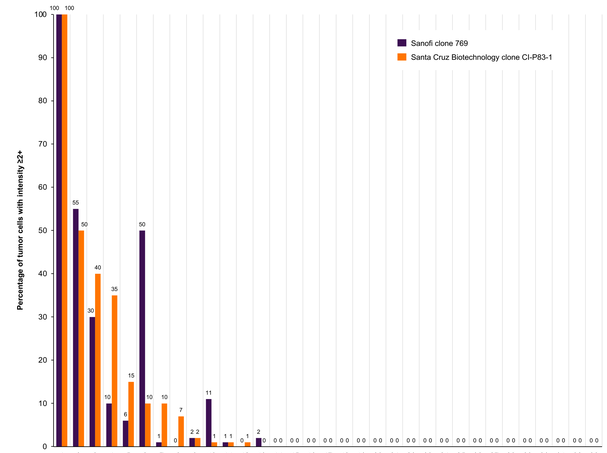


CHO, Chinese hamster ovary; CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; CEACAM6, carcinoembryonic antigen-related cell adhesion molecule 6.

Fit-for-purpose validation in nonsquamous NSCLC tissues

- Plasma membrane scoring for the percentage of CEACAM5 staining intensity ≥2+ in plasma membrane tumors across a panel of nonsquamous NSCLC tissues demonstrated a comparable range of sensitivity for Sanofi clone 769 and Santa Cruz Biotechnology clone CI-P83-1 (**Figure 5**)

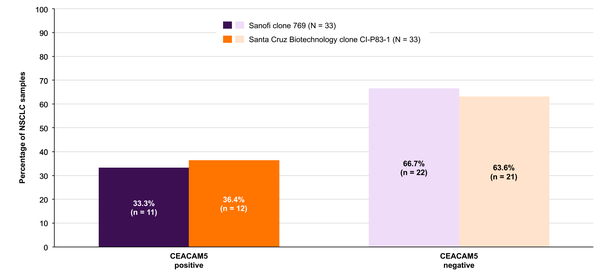
Figure 5. Plasma membrane tumor cells with CEACAM5 intensity ≥2+ in nonsquamous NSCLC tissue samples by antibody.



CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; NSCLC, non-small cell lung cancer.

- Furthermore, Sanofi clone 769 and Santa Cruz Biotechnology clone CI-P83-1 had similar proportions of NSCLC samples that were CEACAM5 positive (≥1% of tumor plasma membrane cells with intensity ≥2+) (**Figure 6**)

Figure 6. CEACAM5 positive and negative nonsquamous NSCLC samples by CEACAM5 status. CEACAM5 positive was defined as intensity ≥2+ in ≥1% of tumor cells; CEACAM5 negative was defined as intensity ≥2+ in <1% of tumor cells.



CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; NSCLC, non-small cell lung cancer.

CONCLUSIONS

- Among 5 commercially available anti-CEACAM5 mAbs tested, the Santa Cruz Biotechnology clone CI-P83-1 performed closest to the previously validated Sanofi clone 769
- Santa Cruz Biotechnology clone CI-P83-1 was appropriately reactive in CEACAM5 expression control cells, highly reactive in CHO cells overexpressing CEACAM5, and lacked cross reactivity in CHO cells overexpressing CEACAM types 1, 6, 7, or 8
- Santa Cruz Biotechnology clone CI-P83-1 had comparable staining intensity results to those of Sanofi clone 769 in nonsquamous NSCLC tissues
- Further testing beyond the fit-for-purpose validation is required to understand the finer differences between the validated Sanofi clone 769 and the Santa Cruz Biotechnology clone CI-P83-1

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DISCLOSURES

All authors declare that they have no disclosures to report.