Trophoblast cell surface antigen 2 (TROP2) is the target of sacituzumab govitecan (SG), an antibody-drug conjugate that was recently approved for previously treated triple negative breast cancer and urothelial carcinomas. In order to learn more about the role of TROP2 for tumor biology and identify other tumor types that might benefit from anti-TROP2 therapies, a comprehensive analysis of TROP2 protein expression across virtually all types of human normal and neoplastic tissues was performed.

**Introduction and Objectives**

Trophoblast cell surface antigen 2 (TROP2) is the target of sacituzumab govitecan (SG), an antibody-drug conjugate that was recently approved for previously treated triple negative breast cancer and urothelial carcinomas. In order to learn more about the role of TROP2 for tumor biology and identify other tumor types that might benefit from anti-TROP2 therapies, a comprehensive analysis of TROP2 protein expression across virtually all types of human normal and neoplastic tissues was performed.

**Tissue microarrays containing 18,563 samples from 150 different tumor types and subtypes as well as 608 samples of 76 different normal tissue types was analyzed by immunohistochemistry.**

Antibody validation by comparison of antibodies. The panels show a complete concordance of staining results obtained by two independent TROP2 antibodies. Using MVA-733R, the stainings show a strong predominantly membranous staining of galbladder epithelium (A) and of intrahepatic bile ducts (B) while staining is less intense and focused on surface epithelial cells and glands (weaker) of the stomach (C) and limited to few interspersed epithelial cells in the colon (D). Using clone AF650, nearly identical stainings are seen in gallbladder (E), liver (F), stomach (G), and the colon (H). The images A-D and E-H were taken from consecutive tissue sections. Due to the polyclonal nature of AF650, background staining is slightly higher than seen for MVA-733R.

**Materials & Methods**

**Immunostaining protocol and controls**
- Antigen retrieval: 5 min at 121°C (autoclave) in pH 7.8 buffer
- Controls:
  - Positive: Strong membranous Trop-2 immunostaining should be seen in bile ducts of the liver.
  - Negative: Liver hepatocytes should not show any Trop-2 immunostaining.

**TROP2 immunostaining examples**

**TROP2 in normal tissues**

TROP2 immunostaining was always membranous and found in many epithelial cell types. The panel shows strong TROP2 positivity of surface epithelial cells of the tonsil (A), urothelium of the urinary bladder (B), and the endometrium (C) as well as in acinar and basal cells of the prostate (D). TROP2 staining is weaker and largely limited to the most apical elements of the surface epithelium in the stomach antrum (E), distal tubuli and the visceral layer of the Bowman capsule of the kidney (F), and intrahepatic bile ducts of the liver (G). TROP2 immunostaining is lacking in colon epithelial cells (H).

**TROP2 in tumor tissues**

Strong, membranous and cytoplasmatic TROP2 immunostaining in a squamous cell carcinoma of the oral cavity (A), a recurrent adenocarcinoma (Gleason 5+6+10) of the prostate (B), a breast cancer of no special type (C), a gastric adenocarcinoma (D), a papillary carcinoma of the thyroid (E), and an adenocarcinoma of the lung (F). TROP2 staining is absent in an epitheloid pleural mesothelioma (G) and a colorectal adenocarcinoma (H).

**Ranking and prognostic value of TROP2 in tumor tissues**

High TROP2 expression was linked to adverse tumor features in colorectal cancer, gastric adenocarcinoma and papillary thyroid cancer. Low TROP2 expression was linked to advanced stage in urothelial carcinoma, high stage and grade and "triple negative receptor status" in breast cancer, as well as with high stage and grade in papillary renal cell carcinomas. No associations were found in clear cell renal cell, ovahan, pancreatic, and endometrium carcinomas.

**Ranking order of TROP2 immuno-staining in cancers. Both the frequency of positive cases (blue dots) and the frequency of strongly positive cases (orange dots) are shown.**

**Conflicts of interest:** The TROP2 antibody clone MVA-733R was provided by MS Validated Antibodies GmbH (owned by a family member of GS).

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