Characterization and prevalence of the novel immunomodulatory protein Siglec-15 expression in tumor cells and macrophages across multiple solid tumor indications

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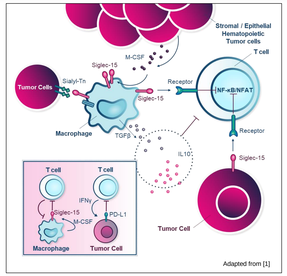
Abstract # 521

Background

- Siglec-15 (S15) is a member of the sialic acid-binding Ig-like lectins (Siglec) family and is a novel target for immunotherapy in cancer
- Siglec-15 has been shown to be expressed on tumor-associated macrophages (TAMs) and tumor cells across multiple solid tumor indications [1]
- Preclinical studies have demonstrated that Siglec-15 suppresses T cell response and blocking the interaction of Siglec-15 with its receptor on T cells releases the immunosuppression [2]
- To characterize the potential of Siglec-15 as a therapeutic target for solid tumors with high unmet need, an immunohistochemistry (IHC) assay was developed with a proprietary antibody to detect Siglec-15
- Siglec-15 RNA was previously shown to be co-expressed with CD274 (PD-L1) in non-small lung cancer (NSCLC) [3] and determination of the prevalence of their protein expression may inform targeting both immune modulatory pathways simultaneously for the treatment of NSCLC

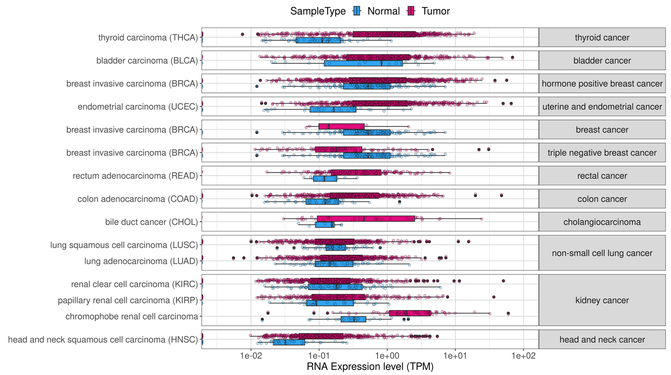
Mechanism of Action of Siglec-15



Methods

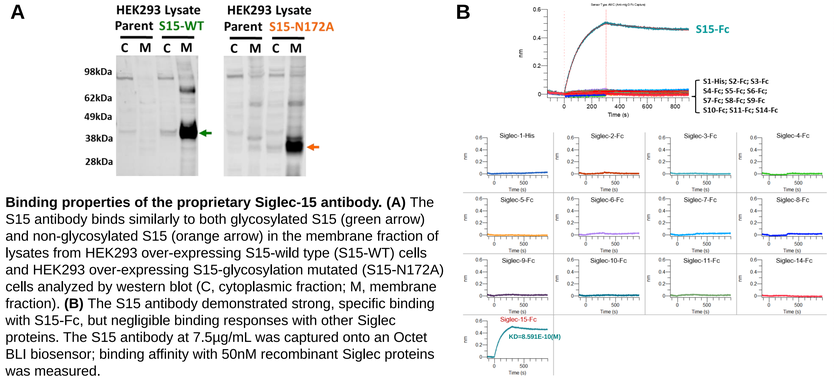
Gene expression data was obtained from The Cancer Genome Atlas (TCGA) through QIAGEN OmicSoft platform to identify tumor types with significantly higher expression of Siglec-15 RNA compared to normal tissue. To confirm protein expression in tumor tissues, a proprietary monoclonal Siglec-15 antibody was generated from a Siglec-15 positive hybridoma to maximize specificity and sensitivity. Antibody binding affinity and specificity were assessed by biolayer interferometry (BLI) for Siglec family proteins. Western blot analyses were performed to detect Siglec-15 protein in cell lines overexpressing wild type and mutant human Siglec-15 at the glycosylation residue N172 [4]. An IHC assay to detect Siglec-15 across cell lines and primary human samples was developed. The IHC assay was optimized on the Dako Link 48 staining platform at a concentration of 0.15 µg/ml. To evaluate Siglec-15 expression patterns and prevalence across cancer indications, whole tissue slides were commercially sourced and stained following an optimized IHC protocol. Slides from approximately 20 individuals per indication were evaluated (bladder n= 19, breast n=20, cholangiocarcinoma n= 22, colon n=22, endometrial n=19, HNSCC n=19, kidney n=20, NSCLC n=29, and thyroid cancers n= 20). To evaluate Siglec-15 expression in normal tissue, a tissue microarray was used with approximately 3 cores from different donors per tissue type. Expression of Siglec-15 was annotated by a board-certified pathologist across commercial human tumor formalin-fixed, paraffin embedded (FFPE) whole tissue samples from each tumor indication and cores from normal tissues. The average number of Siglec-15 positive (S15+) macrophages in a 20x microscopic field within the tumor microenvironment (TME) and the percentage of Siglec-15 positive (S15+) tumor cells at any intensity level was determined for each human tumor tissue sample. PD-L1 expression was assessed in NSCLC samples using the PD-L1 IHC 22C3 pharmDx (Agilent) antibody according to kit instructions. PD-L1 expression was determined by a pathologist using tumor proportion scoring (TPS) methodology. A sample was considered PD-L1+ if the TPS was ≥ 1%. PD-L1+ samples were subdivided into PD-L1 high (TPS ≥ 50%) and PD-L1 low (TPS < 50%) groups.

Increased Siglec-15 gene transcripts observed across multiple primary tumor types compared with normal tissue expression



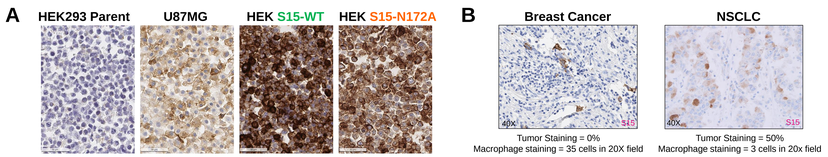
Expression of Siglec-15 mRNA in primary bulk tumors and matched normal tissue. RNA seq data from primary bulk human tumor and matched normal tissue samples from the TCGA database are plotted as transcripts per million (TPM). Siglec-15 is overexpressed in multiple tumor types (shown in pink) compared to normal tissue expression (shown in blue).

The proprietary Siglec-15 antibody selectively binds to Siglec-15 and not other Siglec family members



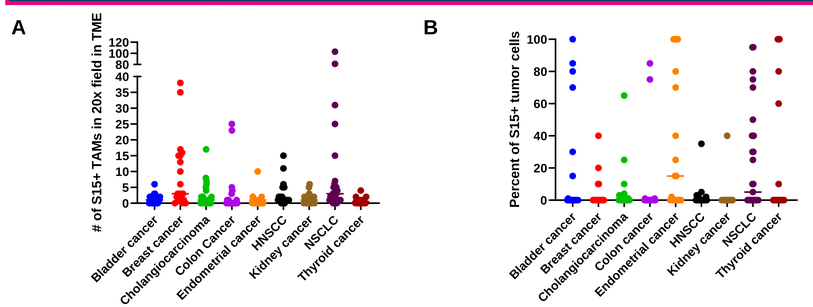
Binding properties of the proprietary Siglec-15 antibody. (A) The S15 antibody binds similarly to both glycosylated S15 (green arrow) and non-glycosylated S15 (orange arrow) in the membrane fraction of lysates from HEK293 over-expressing S15-wild type (S15-WT) cells and HEK293 over-expressing S15-glycosylation mutated (S15-N172A) cells analyzed by western blot (C, cytoplasmic fraction; M, membrane fraction). **(B)** The S15 antibody demonstrated strong, specific binding with S15-Fc, but negligible binding responses with other Siglec proteins. The S15 antibody at 7.5µg/mL was captured onto an Octet BLI biosensor; binding affinity with 50nM recombinant Siglec proteins was measured.

IHC assay development to detect Siglec-15 in tumor tissues



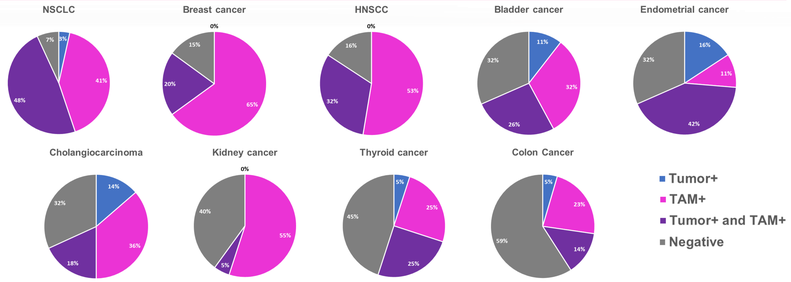
Development of an IHC assay to detect Siglec-15 in FFPE tumor tissue samples. (A) Studies were performed to confirm binding of the S15 antibody to S15 in FFPE cell pellets. The IHC assay detected S15 in a cell line that endogenously expressed S15 (U87MG) and in cell lines overexpressing S15 (HEK S15-WT) and S15 with a mutation at the N-glycosylation site (HEK S15-N172A) but not in the negative control cell line (HEK293 Parent). **(B)** Primary human FFPE tumor tissues were stained with the optimized S15 IHC assay. The predominant cell populations expressing S15 were macrophages and tumor cells. A representative breast cancer sample (left image) indicating S15+ staining in TAMs and a NSCLC sample (right image) indicating S15+ staining in tumor cells.

Siglec-15 is broadly expressed on TAMs and tumor cells across solid tumor types



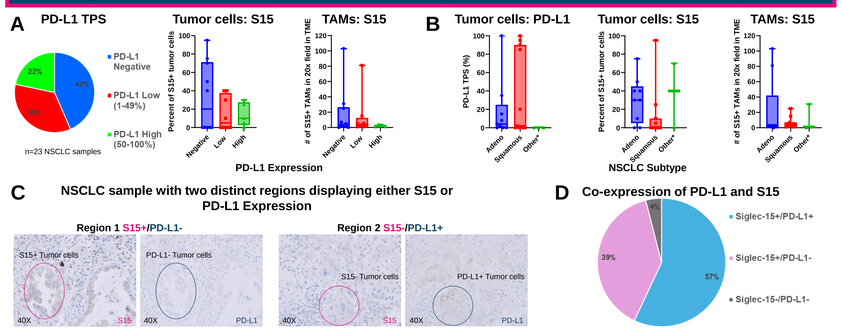
Siglec-15 is broadly expressed in TAMs and tumor cells across multiple solid tumor indications. Primary tumor FFPE samples that were stained with an optimized S15 IHC assay showed differences in prevalence of S15+ cells within and across indications. The average number of S15+ macrophages in a 20x microscopic field within the TME and the percentage of S15+ tumor cells at any intensity level was determined for each human tumor tissue sample. S15 protein was detected in **(A)** TAMs and **(B)** tumors cells across the following indications: bladder cancer, breast cancer, cholangiocarcinoma, colon cancer, endometrial cancer, HNSCC, kidney cancer, NSCLC, and thyroid cancer. Most normal tissues had very few S15+ macrophages and/or some weak expression in epithelium (not shown).

Prevalences of Siglec-15 expression varies across solid tumor indications



The prevalence of Siglec-15+ TAMs and/or tumor cells vary across indications. Each tumor indication was divided into tumor+ (≥1% S15+ tumor cells at any intensity level), TAM+ (≥1 S15+ TAM in a 20x field in the TME at any intensity), tumor+ and TAM+ (≥1% S15+ tumor cells and ≥1 S15+ TAM in a 20x field in the TME at any intensity level) or negative based on the observed S15 staining. NSCLC had the highest rate of S15 positivity across the tumor indications (93%) followed by breast cancer (85%), head and neck cancer (84%), bladder cancer, endometrial cancer, and cholangiocarcinoma (68%), kidney cancer (60%), thyroid cancer (55%), and colon cancer (41%).

Siglec-15 and PD-L1 proteins are co-expressed in NSCLC tumor samples



Co-expression of Siglec-15 and PD-L1 proteins in NSCLC tumor samples. Presence of both S15 and PD-L1 within the same tumor sample supports the potential to target both immune modulatory pathways simultaneously for treatment of NSCLC. **(A)** 57% of NSCLC samples expressed PD-L1 (TPS≥ 1%), 22% with high PD-L1 expression (TPS≥ 50%) and 35% with low PD-L1 expression (TPS <50%). Samples with higher PD-L1 expression tended to have lower S15 expression (tumor cells and TAMs), although not statistically significant. **(B)** Squamous NSCLC tended to have higher PD-L1 expression, while adenocarcinoma NSCLC (Adeno) tended to have higher S15 expression (tumor cells and TAMs), although not statistically significant. *Other: Adenosquamous, large cell or unknown subtype of NSCLC. **(C)** Images from 2 separate regions of the same tumor section which expressed S15 (40% S15+ tumor cells and 3 S15+ TAMs in a 20x field in the TME) and PD-L1 (TPS = 15%). Region 1 shows tumor cells which express S15, but not PD-L1 and region 2 shows tumor cells which express PD-L1 but not S15. **(D)** All PD-L1+ samples had S15+ staining in either tumor cells, macrophages, or both. One sample was negative for both S15 and PD-L1.

Conclusions

- Siglec-15 protein was found to be broadly expressed by TAMs and tumor cells within a range of tumor indications with NSCLC having the highest rate of positivity.
- Detection of both Siglec-15 and PD-L1 proteins in more than half of the NSCLC samples tested support the potential to target both immunomodulatory pathways in combination for the treatment of NSCLC.
- The ability of the IHC assay to specifically detect S15 on tumor cells and TAMs position it as a potential patient selection assay to facilitate clinical studies involving S15 targeting therapeutics.
- An investigational S15 antibody, PYX-106, is currently being tested in a Phase I trial (NCT05718557) across a broad range of tumor indications including NSCLC, breast cancer, HNSCC, thyroid cancer, endometrial cancer, bladder cancer, kidney cancer, colon cancer and cholangiocarcinoma.

References

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[3] Watkins K, Dias L, Crochiere M, Pinkas J; Abstract 1373: Gene expression correlation of immune checkpoint molecules Siglec-15 and PD-L1 varies widely by cancer indication. Cancer Res 15 March 2024; 84 (6_Supplement): 1373.
[4] Wang YL, Wei MB, Zhao WW, Feng LL, Yin XK, Bai SM, Wan XB, Hung MC, Zou AZ, Wang MH, Zheng J, Qin C, Fan XJ. Glycosylation of Siglec15 promotes immunoescape and tumor growth. Am J Cancer Res. 2021 May 15;11(5):2291-2302.

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