

## Supplementary Data

### **Tumor-Associated CD75s- and iso-CD75s-Gangliosides are Potential Targets for Adjuvant Therapy in Pancreatic Cancer**

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## **Table of Contents:**

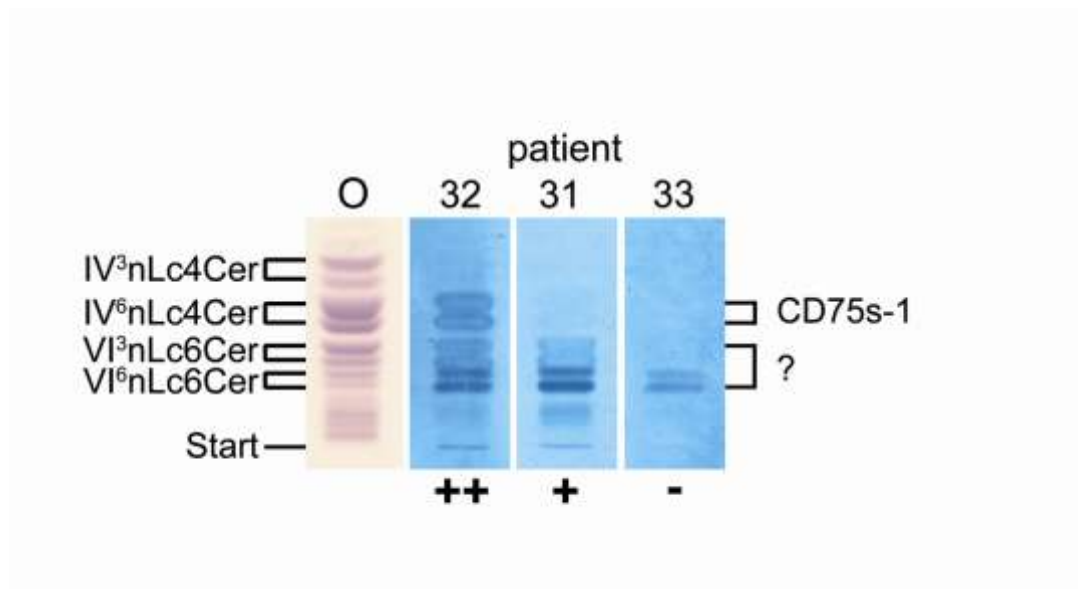
**Figure S1.** TLC overlay assay detection of anti-CD75s-1-ganglioside antibodies in sera from patients with pancreatic carcinoma.

**Table S1:** Pathological data of pancreatic carcinoma and tissue wet weights of normal and malignant tissues of the pancreas

**Table S2:** Molecular ions obtained from immunostained reference CD75s- and iso-CD75s-gangliosides in the positive ion mode by direct TLC-IR-MALDI-MS

**Table S3:** Synopsis of ganglioside binding specificities of polyclonal AB2-3 and AB2-6 antibodies and of viscumin and its recombinant pendant rViscumin

**Table S4:** Anti-CD75s-1-ganglioside antibodies in serum samples of patients with pancreatic carcinoma



**Figure S1.** TLC overlay assay detection of anti-CD75s-1-ganglioside antibodies in sera from patients with pancreatic carcinoma. Comparative TLC runs were performed with 10  $\mu$ g of reference gangliosides for orcinol stain (O) and the TLC overlay detections using patients sera, respectively. Three examples of sera from patients 32, 31, and 33, corresponding to moderate positive (++) , weak positive (+), and negative (-) anti-CD75s-1-ganglioside binding, respectively, are shown (see Supplementary [Table S4](#)). For pathological data of tumor patients and structures of reference gangliosides see Supplementary Table S1 and Supplementary Table S2, respectively. The structural interpretation of positive gangliosides which chromatograph beneath CD75s-1-gangliosides and marked with question marks remains elusive.

**Table S1: Pathological data of pancreatic carcinoma and wet weights of normal and malignant tissues of the pancreas**

patient	pT*	pN <sup>†</sup>	pM <sup>‡</sup>	UICC stage <sup>§</sup>	G <sup>  </sup>	tissue wet weight [mg]	
						normal	tumor
1	3	1	1	IVB	2	44.1	75.4
2	3	1	X	X	3	58.3	85.4
3	3	1	X	X	2	20.2	40.4
4	3	1	0	III	X	55.8	74.9
5	2	1	0	III	2	110.9	59.1
6	3	0	X	X	2	63.8	53.2
7	4	1(4/8)	X	X	2	143.5	153.0
8	3	1	X	X	2-3	32.5	71.2
9	4	1(9/24)	X	X	2	77.6	119.6
10	3	1	1	IVB	2	110.1	153.4
11	3	1(2/20)	X	X	2	27.7	88.3
12	3	1(2/19)	X	X	2	18.0	27.6
13	3	1	X	X	2	60.4	54.7
14	3	1(8/27)	X	X	3	28.4	45.1
15	3	1(2/10)	X	X	3	92.6	40.6
16	3	0(0/17)	X	X	2	12.8	77.9
17	3	1	0	III	3	23.5	31.5
18	3	1	0	III	4	47.4	80.5
19	3	1	0	III	2	22.9	39.2
20	3	1	0	III	2	38.5	59.8
21	3	1	0	III	3	27.2	41.6
22	3	1	0	III	3	23.9	19.0
23	1	0	0	I	2	82.1	51.9
24	3	0	0	II	3	18.6	26.3
25	3	1	0	III	2	63.2	60.5
26	3	0	0	II	3	18.2	42.8
27	3	0	0	II	2	8.5	45.1
28	3	0	0	II	3	17.4	25.7
29	3	1	0	III	3	28.4	68.8
30	3	1	1	IVB	3	30.8	36.4
31	3	0	0	II	2	78.1	63.1
32	3	1	0	III	2	36.3	47.6
33	3	1	1	IVB	2	78.0	36.4
34	3	1	0	III	3	36.3	65.1
35	3	1	0	III	3	28.0	69.9
36	3	1	0	III	2	20.0	43.0
37	3	0	0	II	3	22.0	115.1
38	2	X	X	X	2	197.5	75.8

\* T = The extent of the primary tumor. TX: Primary tumor cannot be assessed, T0: No evidence of primary tumor, Tis: Carcinoma in situ; T1: Tumor limited to the pancreas,  $\emptyset \leq 2$  cm, T2: Tumor limited to the pancreas,  $\emptyset > 2$  cm, T3: Tumor extends directly into any of the following: duodenum, bile duct, peripancreatic tissues, T4: Tumor extends directly into any of the following: stomach, spleen, colon, adjacent large vessels

† N = The absence or presence and extent of regional lymph node metastasis. NX: Regional lymph nodes cannot be assessed, N0: No regional lymph node metastasis, N1: Regional lymph node metastasis

‡ M = The absence or presence of distant metastasis. MX: Distant metastasis cannot be assessed, M0: No distant metastasis, M1: Distant metastasis

§ Stage grouping. Stage 0: Tis N0 M0, Stage I: T1 N0 M0/ T2 N0 M0, Stage II: T3 N0 M0, Stage III: T1 N1 M0/T2 N1 M0/ T3 N1 M0, Stage IVA: T4 Any N M0, Stage IVB: Any T Any N M1, X: Stage cannot be assessed

|| Histopathological grading. G1: well differentiated, G2: moderately differentiated, G3: poorly differentiated, G4: undifferentiated, X: Histopathological grading cannot be assessed

**Table S2: Molecular ions obtained from immunostained reference CD75s- and iso-CD75s-gangliosides in the positive ion mode by direct TLC-IR-MALDI-MS\***

CD no. <sup>†</sup>	structure <sup>‡</sup>	[M+2Na-H] <sup>+</sup> m/z (monoisotopic) calculated/detected
iso-CD75s-1	IV <sup>3</sup> Neu5Ac-nLc4Cer (d18:1, C24:0)	1674.94/1674.93
	IV <sup>3</sup> Neu5Ac-nLc4Cer (d18:1, C24:1)	1672.93/1672.92
	IV <sup>3</sup> Neu5Ac-nLc4Cer (d18:1, C16:0)	1562.82/1562.80
CD75s-1	IV <sup>6</sup> Neu5Ac-nLc4Cer (d18:1, C24:1)	1672.93/1672.89
	IV <sup>6</sup> Neu5Ac-nLc4Cer (d18:1, C16:0)	1562.82/1562.79
iso-CD75s-2	VI <sup>3</sup> Neu5Ac-nLc6Cer (d18:1, C24:0)	2040.07/2040.04
	VI <sup>3</sup> Neu5Ac-nLc6Cer (d18:1, C24:1)	2038.06/2038.03
	VI <sup>3</sup> Neu5Ac-nLc6Cer (d18:1, C16:0)	1927.95/1927.94
CD75s-2	VI <sup>6</sup> Neu5Ac-nLc6Cer (d18:1, C24:1)	2038.06/2038.04
	VI <sup>6</sup> Neu5Ac-nLc6Cer (d18:1, C16:0)	1927.95/1927.91

\* only dominant disodiated [M+2Na-H]<sup>+</sup> molecular ions are listed; orcinol stains (O) and TLC immunostains (AB2-6 and AB2-3) of the reference CD75s- and iso-CD75s-gangliosides are shown in Figure 1A and 3A, respectively; iso-CD75s-1- and iso-CD75s-2-gangliosides with C24:0 and C24:1 fatty acids chromatograph inseparably in single TLC bands, respectively

<sup>†</sup> CD: cluster of differentiation (13)

<sup>‡</sup> structures of CD75s- and iso-CD75s-1-gangliosides are depicted in Figure 1B and 3B, respectively; nLc4Cer (type II core): Galβ4GlcNAcβ3Galβ4Glcβ1Cer, nLc6Cer (type II core with one repeat): Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer (14)

**Table S3: Synopsis of ganglioside binding specificities of polyclonal AB2-3 and AB2-6 antibodies and of viscumin and its recombinant pendant rViscumin\***

CD no. <sup>†</sup>	AB2-3	AB2-6	viscumin/rViscumin	references
iso-CD75s-1	+	-	-	14, 24
Iso-CD75s-2	+	-	-	14, 24
CD75s-1	-	+	+	14-17,24
CD75s-2	-	+	+	14-17,24

\* structures of CD75s- and iso-CD75s-gangliosides are depicted in Figure 1B and 3B, respectively, and listed in Supplementary Table S2

<sup>†</sup> CD: cluster of differentiation (13)

**Table S4: Anti-CD75s-1-ganglioside antibodies in serum samples of patients with pancreatic carcinoma\***

category*	percentage [%]	patient
<b>++</b>	<b>31.6</b>	20
		21
		24
		25
		27
		32
<b>+</b>	<b>26.3</b>	22
		23
		28
		31
		35
<b>-</b>	<b>42.1</b>	17
		18
		19
		26
		29
		30
		33
		34

\* in toto 19 serum samples, which have been taken before surgery, were available from the cohort of 38 patients (see Table 1 and Supplementary Table S1)

† appearance of immunopositive bands in TLC overlay assays graded for serum anti-CD75s-1 ganglioside antibodies as ++ moderate positive reaction, + weak positive reaction and - no reaction (negative)