Identification of Tumor-Marker Gangliosides for Early Cancer Detection: A Multi-Platform Approach

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Abstract

Tumor-marker gangliosides (TMGs) have enormous potential for early-stage cancer detection as diagnostic biomarkers despite being relatively understudied in this context. AOA Dx is focused on the analytical development of three separate platforms for the quantitation of TMGs in human serum, particularly disialogangliosides GD2 and GD3. The development of these platforms for TMG quantitation may allow for the identification of diagnostic signatures for early-stage cancer detection.

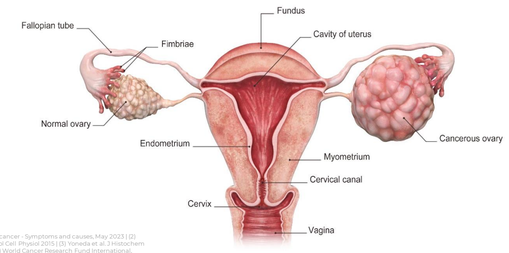
First, we are developing a pipeline of antibody-based assays to target GD2 and GD3. To aid in development of multiple immunoassays, we have collected specificity and affinity data on several commercial and proprietary antibodies targeting GD2 and GD3 using glycoarrays. These data confirm that GD2 and GD3 antibodies with negligible levels of cross-reactivity and high target-affinity can be designed and used in multiple platforms.

We are currently using two immune-based platforms to target TMGs. We have developed an enzyme-linked immunosorbent assay (ELISA) to quantify the levels of GD2 and GD3 in complex matrices including human serum and plasma, cell lines, exosomes, and liposomes and we have implemented high-performance thin-layer chromatography (HPTLC) as a tool to detect individual TMGs GD2 and GD3, as well as total gangliosides in each of the above matrices. These platforms allow for high-throughput analysis of samples for TMG quantitation. Additionally, we are applying both targeted and untargeted mass spectrometry analyses and have identified multiple TMGs, including GD2 and GD3 species characterized by unique lipid tails. These analyses were performed using human serum from confirmed cancer diagnoses. Notably, the serum of melanoma and ovarian cancer patients exhibited significantly increased levels of several ganglioside species compared to age-matched healthy serum, further highlighting the potential of TMGs as a promising avenue for cancer diagnosis.

Further experiments will focus on refining our ELISA and HPTLC platforms. We will also expand the demographic representation of our human serum sample cohort to confirm the initial findings from our mass spectrometry experiments and continue to use this platform to identify additional TMGs of interest.

Together, **AOA Dx is advancing the development of a multi-platform approach for the detection of TMGs, with a primary focus on GD2 and GD3**. Our objective is the identification of a **diagnostic disease signature for early-stage cancer detection**. The validation of this type of diagnostic panel would allow for expedited diagnosis and treatment of cancers currently diagnosed at late stages, ultimately reducing healthcare costs and increasing survival rates. Future research will aim to validate these biomarkers in independent prospective studies.

Ovarian cancer (OC) is deadly, but symptomatic



(1) Mayo Clinic, Ovarian cancer - Symptoms and causes, May 2023 | (2) Yeung et al, Am J Physiol Cell Physiol 2015 | (3) Yoneda et al. J Histochem Cytochem. 2012 Jan | (4) World Cancer Research Fund International, Ovarian Cancer Statistics, Apr 2022

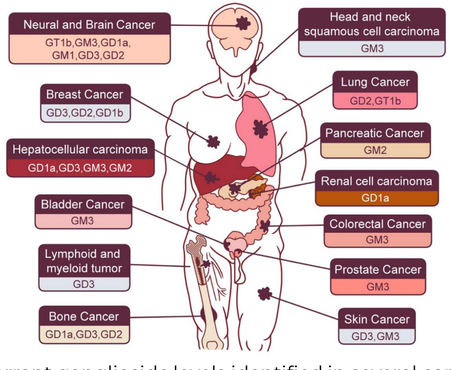
- 8th most common cancer in women worldwide[1], dubbed the "silent killer."[4]
- 94% experience symptoms, but symptoms are vague and often attributed to more common conditions[2].
- >70% cases diagnosed at stages III or IV, reducing 5-year survival rates.[3]

Survival rate could be improved by early diagnosis

- Most common path to diagnosis combines transvaginal ultrasound and serum CA-125[5]
- Biomarkers (ex. CA-125) show low clinical sensitivity[6]
- CA-125 is **not** elevated in…
  - 50% of early-stage disease
  - 20% of advanced stage disease
- <50% diagnosed within 1 month of first doctor visit[7]
- Average time to diagnosis = 8 months

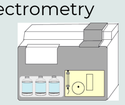
Ovarian Cancer Research Alliance, May 2022 | (2) Carter et al. Female Patient (Parsippany). 2011; 36(4): 30–35. | Mayor et al. BMJ. 2018;363:k4419 | (3) World Ovarian Cancer Coalition, Too late to treat – average time to an ovarian cancer diagnosis is almost 8 months, May 2022

Gangliosides: powerful novel biomarkers

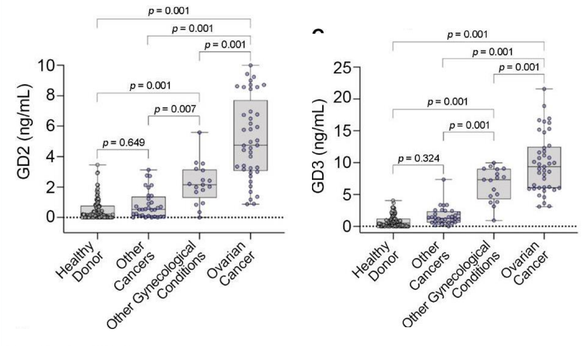


- Aberrant ganglioside levels identified in several cancers
- Heterogenous levels/distribution indicate unique disease signature
- Gangliosides relatively low in healthy serum
- Tumor marker gangliosides (TMGs) increased in cancer

Multiple, orthogonal platforms for TMG analysis

Platform	Use
ELISA 	• Quantitation of 1-3 gangliosides in a sample • High throughput: 100+ samples per day • Used to identify samples with aberrant ganglioside expression
Thin Layer Chromatography 	• Semi-quantitation of total gangliosides (chromagen staining) or single gangliosides (immuno-overlay) • Used in conjunction with ELISA or MS to confirm presence or absence of gangliosides
Mass Spectrometry 	• High-throughput detection and quantitation of multiple ganglioside species • Used to characterize disease cohorts and identify which gangliosides show aberrant expression in different disease states

Quantitative ELISA shows elevated GD2, GD3 in serum



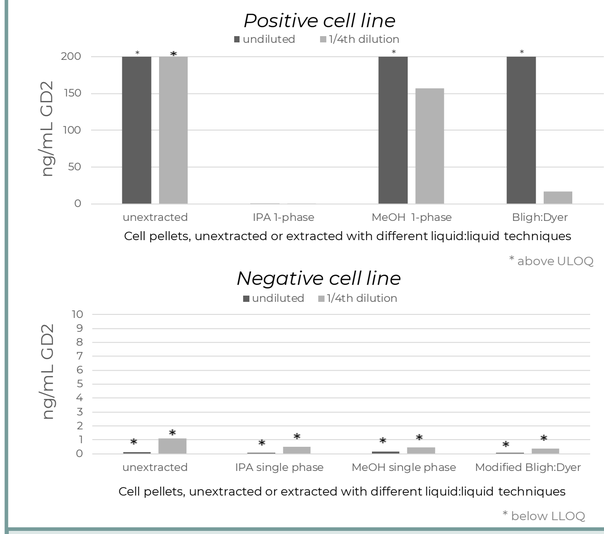
Galan et al. Front Oncol. 2023 Apr 14.

GD2 antibody shows sensitivity, specificity to GD2

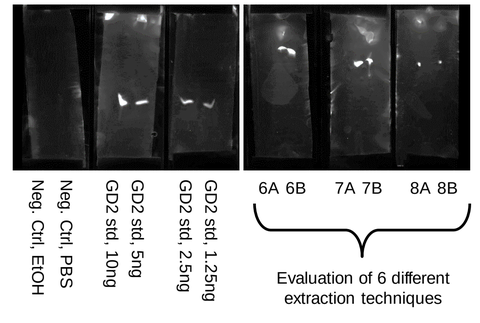
- Qualitative glycoarray to screen Abs for affinity, specificity to GD2 *performed by Hugh Willison and Susan Halstead.*
- Using in-house printed array chips, antigen is printed onto slides into which monoclonal antibody is incubated. Bound antibody detected using fluorescent antibody.

GM1	GM2	GM3	GM4	MtOH
PS	GD1a	GD1b	GT1a	GT1b
GQ1b	GD3	MtOH	GD2	GD2
GalNAc-GD1a	SGPG	LM1	GalC	Chol
MtOH	Sulph	SM	GA1	GT1a-α

Cells known to highly express GD2 and negative controls confirmed using GD2 ELISA



GD2 can be detected using immune-overlay TLC in a known-positive GD2 cell line



Initial targeted HPLC-MS shows increased GD2, GD3 in ovarian cancer