CD24 gene amplification associates with poor survival outcomes across cancer indications

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Background

- CD24 (cluster of differentiation 24) is a highly glycosylated protein overexpressed on the surface of many cancer cells[1]
- By interacting with the macrophage receptor protein, Siglec-10 (sialic-acid-binding Ig-like lectin-10), CD24 acts as a "don't eat me" signal to protect cancer cells from being engulfed by tumor-associated macrophages[1]
- *CD24* overexpression is most frequent in solid tumors, where it can be >100-fold compared with normal tissue;[1,2] however, the clinical implications of this overexpression have not been fully elucidated
- We analyzed *CD24* copy number (CN), mRNA, and clinical outcomes across all available cancer types in The Cancer Genome Atlas (TCGA)
- As small lung cell cancer (SCLC) data were not available in TCGA, we analyzed *CD24* CN and mRNA in SCLC-derived cell lines from DepMap
- Using DNAscope and immunohistochemistry, we evaluated *CD24* CN and protein expression, respectively, in primary ovarian serous carcinoma samples

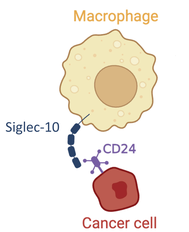
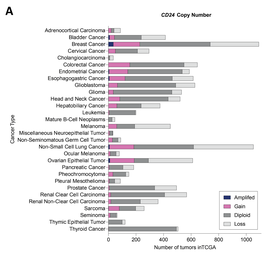
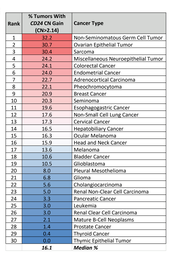


Figure 1. *CD24* CN gain at *chr6q21* is prevalent in many TCGA primary cancers

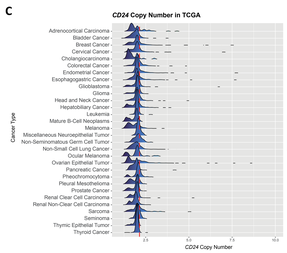


A. *CD24* CN alterations in various cancer types in TCGA. Amplified, CN>5; Gain, CN 2.14–5; Diploid, CN 1.87–2.14; Loss, CN<1.87.



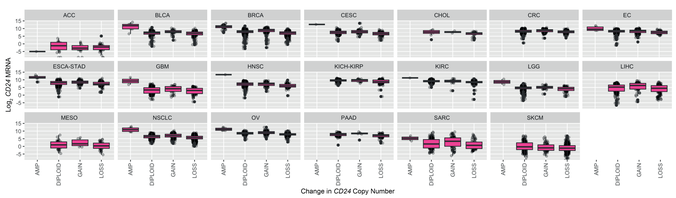
Rank	% Tumors With *CD24* CN Gain (CN>2.14)	Cancer Type
1	32.2	Non-Seminomatous Germ Cell Tumor
2	30.7	Ovarian Epithelial Tumor
3	30.4	Sarcoma
4	24.2	Miscellaneous Neuroepithelial Tumor
5	24.1	Colorectal Cancer
6	24.0	Endometrial Cancer
7	22.7	Adrenocortical Carcinoma
8	22.1	Pheochromocytoma
9	20.9	Breast Cancer
10	20.3	Seminoma
11	19.6	Esophagogastric Cancer
12	17.6	Non-Small Cell Lung Cancer
13	17.3	Cervical Cancer
14	16.5	Hepatobiliary Cancer
15	16.3	Ocular Melanoma
16	15.9	Head and Neck Cancer
17	13.6	Melanoma
18	10.6	Bladder Cancer
19	10.5	Glioblastoma
20	8.0	Pleural Mesothelioma
21	6.8	Glioma
22	5.6	Cholangiocarcinoma
23	5.0	Renal Non-Clear Cell Carcinoma
24	3.3	Pancreatic Cancer
25	3.0	Leukemia
26	3.0	Renal Clear Cell Carcinoma
27	2.1	Mature B-Cell Neoplasms
28	1.4	Prostate Cancer
29	0.4	Thyroid Cancer
30	0.0	Thymic Epithelial Tumor
	16.1	***Median %***

B. Ranked list of % frequency of *CD24* CN gain in various cancer types in TCGA.



C. Distribution of *CD24* CN status in various cancer types

Figure 2. *CD24* CN gain correlates with mRNA expression in many cancer types



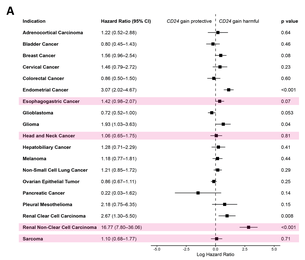
Alterations in *CD24* CN versus *CD24* mRNA expression in TCGA by indication.

Amplified, *CD24* CN>5; Gain, *CD24* CN 2.14–5; Diploid, *CD24* CN 1.87–2.14; Loss, CN<1.87.

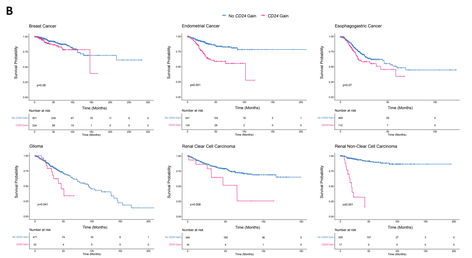
Conclusions

- *CD24* gene amplification status may be a potential biomarker and have prognostic value for various cancers, as *CD24* CN gain
  - is prevalent across many primary cancers and correlates with increased CD24 protein expression
  - may be associated with poorer survival outcomes for patients with breast, endometrial, esophagogastric, and renal cancers, as well as gliomas
  - may be a predictor of worse survival for patients with luminal A breast cancer and uterine corpus endometrial carcinoma
  - is common and correlates with increased mRNA expression in SCLC-derived cell lines
- Representative samples of primary ovarian serous carcinoma indicate that *CD24* gene amplification is specific to cancer cells, and correlates with increased CD24 protein expression

Figure 3. *CD24* CN gain is associated with trends in poorer survival in some tumor types



A. Forest plot showing hazard ratio and statistical analysis for survival across tumor types, based on *CD24* CN alteration (pink boxes indicate p<0.05).

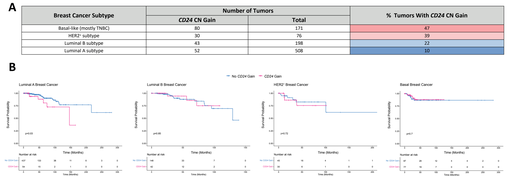


B. Overall Kaplan–Meier survival plots for tumors according to presence or absence of *CD24* CN gain.

Figure 4. *CD24* CN gain is highly enriched in samples of basal-like breast cancer and uterine carcinosarcoma, and is associated with poorer survival in luminal A breast cancer and UCEC

A

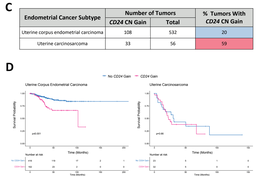
Breast Cancer Subtype	Number of Tumors		% Tumors With *CD24* CN Gain
	CD24 CN Gain	Total	
Basal-like (mostly TNBC)	80	171	47
HER2⁺ subtype	30	76	39
Luminal B subtype	43	198	22
Luminal A subtype	52	508	10



A. Level of *CD24* CN gain enrichment in basal, HER2⁺, and luminal breast cancer subtypes. **B.** Kaplan–Meier survival plots for breast cancer subtypes according to *CD24* CN gain.

C

Endometrial Cancer Subtype	Number of Tumors		% Tumors With *CD24* CN Gain
	CD24 CN Gain	Total	
Uterine corpus endometrial carcinoma	108	532	20
Uterine carcinosarcoma	33	56	59



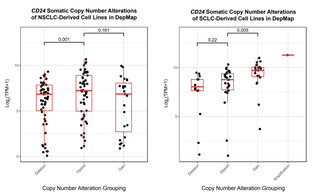
C. Level of *CD24* CN gain enrichment in endometrial cancer subtypes (uterine corpus endometrial carcinoma and uterine carcinosarcoma). **D.** Kaplan–Meier survival plots for endometrial cancer subtypes according to *CD24* CN gain.

Figure 5. Focal *CD24* CN gain is prevalent in small cell lung cancer-derived cancer cell lines in DepMap, and correlates with *CD24* mRNA expression

B

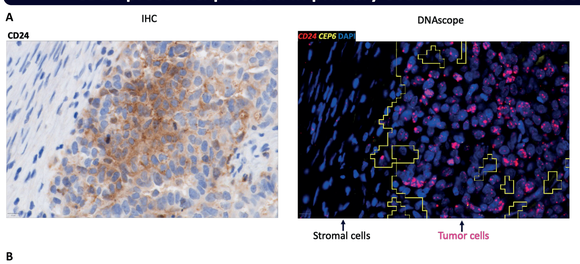
Cancer Cell Line Origin	% Frequency of *CD24* CN Gain (CN>2.14)	% Frequency of *CD24* Focal Amplification	% Frequency of Aneuploidy
SCLC (N=84)	36.9%	10.7%	29.8%
NSCLC (N=148)	19.0%	3.38%	17.6%

A. Loci of *CD24*, *KHDRBS2*, and *PTPRK* on chromosome 6 used in the *CD24* CN alteration analysis: gain was considered focal when the *CD24* locus was amplified, and adjacent proximal and distal regions, as indicated by *KHDRBS2* and *PTPRK*, were unamplified.
B. Percentage *CD24* focal gains in SCLC and NSCLC cell lines.

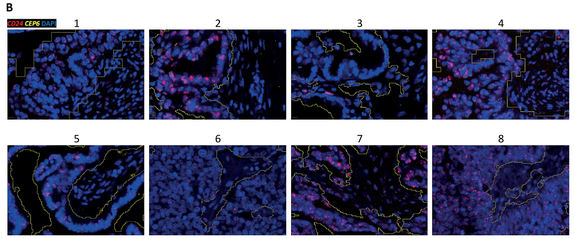


C. Correlation between *CD24* CN and CD24 mRNA in SCLC and NSCLC cell lines.

Figure 6. *CD24* gene amplification is cancer cell-specific, and correlates with increased CD24 protein expression in primary ovarian serous adenocarcinoma samples



A. Representative sample of CD24 protein expression measured by IHC (left panel; brown stain) and *CD24* gene amplification, measured by DNAscope (right panel; pink fluorescence). IHC and DNAscope staining protocols were performed on different, but very closely sectioned FFPE slides from the same tumor. Cell nuclei are visible as blue hematoxylin staining in IHC, and blue DAPI staining in DNAscope. Tumor cells are visible as round nuclei, and adjacent stromal cells are visible as elongated nuclei. Cellular segmentation is annotated by yellow lines.



B. *CD24* gene amplification in five out of eight tumor cell samples from patients with ovarian serous adenocarcinoma. Cell nuclei are visible as blue DAPI staining. Cellular segmentation is annotated by yellow lines.

Data generated by iCura Diagnostics

Abbreviations: ACC: adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CD24, cluster of differentiation 24; CHOL, cholangiocarcinoma; CN, copy number; CRC; colorectal cancer; DAPI, 4',6-diamino-2-phenylindole; EC, endometrial cancer; ESCA-STAD, esophageal and stomach adenocarcinoma; FFPE, formalin-fixed paraffin-embedded; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; KHDRBS2, KH RNA binding domain containing, signal transduction associated 2; KICH-KIRP, kidney chromophobe-kidney renal papillary cell carcinoma; KIRC, kidney renal clear cell carcinoma; LGG, low grade glioma; LIHC, liver hepatocellular carcinoma; MESO, mesothelioma; mRNA, messenger ribonucleic acid; NSCLC, non-small cell lung carcinoma; OV, ovarian serous cystadenocarcinoma; PTPRK, protein tyrosine phosphatase receptor type K; SARC, sarcoma; SCLC, small cell lung cancer; Siglec-10, sialic-acid-binding Ig-like lectin-10; SKCM, skin cutaneous melanoma; TCGA, The Cancer Genome Atlas; TNBC, triple-negative breast cancer; UCEC, uterine corpus endometrial carcinoma.

References: 1. Barkal AA, *et al*. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature*. 2019;572(7769):392-396; 2. Tang Z, *et al*. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res*. 2017;45(W1):W98-W102.

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