

**Kinase and phosphatase inhibition as a signal transduction-centric approach to adjunctive chemotherapies for the treatment of gynecologic cancers: DCTPP1.**

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Men and women differ in large part resulting from the presence or absence of gynecologic organs including the ovaries, the uterus, the lining of which is known as the endometrium, and the entry to the uterus (womb), the cervix (1), all of which are targets for development of malignancies (2-7); vulvar cancer is less common (8). Cancer, or unrestricted cell division which develops into a tumor after remaining unchecked, evades active cell death and cell cycle control mechanisms, sustaining its survival through growth factor signals (9) which are transduced to the nucleus to activate and repress transcription of genes (10, 11). These signals are transduced between the plasma membrane and the nucleus by the action of kinases and phosphatases, enzymes that utilize conformational changes and a catalytic site to translate a signal from the extracellular environment to a change in gene expression. Catalytic sites are pharmacologically targetable, and small molecule targeting of the appropriate kinase or phosphatase target will disrupt or block this transduction (12, 13). We utilized whole transcriptome technologies (14, 15) to identify and validate kinase and phosphatase targets in gynecologic oncology, including cancer of the ovary, or epithelial ovarian cancer. Here we describe a differentially expressed, up-regulated, and catalytically available phosphatase target in epithelial ovarian cancer: DCTPP1.

**Keywords:** kinase, phosphatase, signal transduction, gynecologic oncology, therapeutic targets in cancer, chemical biology, systems biology of human cancer.

We utilize genomic and transcriptomic technologies to study the genomic sequences (DNA), the transcriptome (RNA), the proteome (total collection of proteins in the cell) and epigenetic modification (eg., CpG-DNA) of human cancer (16-20). This includes the primary tumor, the source of the transformation - like mutant variants of p53 - cancer subtypes, like adeno and squamous forms of non-small cell lung cancer, metastasis to distant sites, including the lymph nodes, the lungs, the liver, the brain, and bones, and the circulating tumor stem cell, which emerges from the primary tumor, disseminates through the body and establishes a novel entity in a separate organ (21-26).

Our hypothesis and working strategy dictates that in the short-run, disease complications and therapeutic limitations are best managed using identification of therapeutic targets by whole transcriptome differential expression analyses (subtraction analysis), administered in combination with FDA approved broad-spectrum chemotherapeutics like CDK4/6 inhibitors, and in the long-run, will be enhanced and fully complemented by reverse genetic screening strategies (27, 28) to blindly identify disease-specific, subtype-specific and metastasis-specific therapeutic vulnerabilities, fully augmented by novel immunotherapy approaches. Here we describe a phosphatase therapeutic target identified through rigorous study of the ovarian tumor transcriptome - a phosphatase target that is up-regulated and catalytically available: DCTPP1.

## Results

**Figure 1:** DCTPP1 is differentially expressed in ovarian cancer.

### I. Primary tumors of the ovary from humans with epithelial ovarian cancer.

$n=3$  normal ovarian tissue

$n=8$  primary tumors (ovary; human)

ID	<i>p</i> -value	t	B	logFC	Gene	Rank	%DE
A_23_P33613	8.64E-04	-4.4285739	-0.53156	-1.23465187	DCTPP1	1390/40462	96.6

Through quantitative comparison of total transcription in the normal ovarian tissue and in primary tumors of humans with epithelial ovarian cancer (14), we discovered differential expression of dCTP pyrophosphatase 1, encoded by *DCTPP1* in epithelial ovarian cancer in humans (**Chart 1**). The expression of DCTPP1 changed more than 95% of the human ovarian tumor transcriptome when considering all transcripts whose expression was measured - in this case, 40,462 transcripts ("Rank"). Note the negative fold-change indicating increased quantity of DCTPP1 messenger RNA in ovarian tumors, demonstrating up-regulation of DCTPP1 during transformation of the ovary.

### II. Primary tumors of the ovary from humans with ovarian cancer.

$n=3$  normal ovarian tissue

$n=40$  primary tumors (ovary; human)

ID	<i>p</i> -value	t	B	logFC	Gene	Rank	%DE
8000884	1.06E-01	-1.6493784	-5.2140092	-0.5795223	DCTPP1	10271/29088	64.7

Through quantitative comparison of total transcription in the primary tumors of humans with epithelial ovarian cancer relative to normal ovarian tissue, in a second microarray dataset (15) from independent investigators, we validated differential expression of DCTPP1 in epithelial ovarian cancer in humans (**Chart 2**). The expression of DCTPP1 here changed more than nearly 65% of the human ovarian tumor transcriptome when considering all transcripts whose expression was measured - in this case, 29,088 transcripts ("Rank"). Note the negative fold-change indicating increased quantity of DCTPP1 messenger RNA in ovarian tumors, demonstrating up-regulation of DCTPP1 during transformation of the ovary.

Thus, differential and increased expression of DCTPP1 defines the transcriptional landscape of epithelial ovarian cancer in humans.

## Discussion

Adjunctive treatments in medical oncology limit the emergence of resistant tumor clones during treatment with a second agent (whether neoadjuvantive chemotherapy or a targeted therapy). Small molecule inhibitors of DCTPP1 phosphatase, once evaluated for toxicity and safety, can immediately be tested for efficacy in patients with epithelial ovarian cancer, with the goal of identifying the most effective phosphatase inhibitors for management of gynecologic malignancies. An approach that combines kinase and phosphatase targeting with a recently described multi-catalytic strategy that targets dNTP synthesis, replication of the daughter strand and activity at the spindle at anaphase, likely delivered in conjunction with standard chemotherapies and drug resistance pump inhibitors in resistant cases, is most likely to be most effective in limiting tumor clone resistance and disease (29).

## References

1. Hacker, N.F., Gambone, J.C. and Hobel, C.J., 2015. *Hacker & Moore's essentials of obstetrics and gynecology*. Elsevier Health Sciences.
2. Matulonis, U.A., Sood, A.K., Fallowfield, L., Howitt, B.E., Sehouli, J. and Karlan, B.Y., 2016. Ovarian cancer. *Nature reviews Disease primers*, 2(1), pp.1-22.
3. Veneziani, A.C., Gonzalez-Ochoa, E., Alqaisi, H., Madariaga, A., Bhat, G., Rouzbahman, M., Sneha, S. and Oza, A.M., 2023. Heterogeneity and treatment landscape of ovarian carcinoma. *Nature Reviews Clinical Oncology*, 20(12), pp.820-842.
4. Makker, V., MacKay, H., Ray-Coquard, I., Levine, D.A., Westin, S.N., Aoki, D. and Oaknin, A., 2021. Endometrial cancer. *Nature reviews Disease primers*, 7(1), p.88.
5. Urick, M.E. and Bell, D.W., 2019. Clinical actionability of molecular targets in endometrial cancer. *Nature Reviews Cancer*, 19(9), pp.510-521.
6. Frazer, I.H., 2004. Prevention of cervical cancer through papillomavirus vaccination. *Nature Reviews Immunology*, 4(1), pp.46-55.
7. Alfaro, K., Maza, M., Cremer, M., Masch, R. and Soler, M., 2021. Removing global barriers to cervical cancer prevention and moving towards elimination. *Nature Reviews Cancer*, 21(10), pp.607-608.
8. Li, Z., Liu, P., Wang, Z., Zhang, Z., Chen, Z., Chu, R., Li, G., Han, Q., Zhao, Y., Li, L. and Miao, J., 2023. Prevalence of human papillomavirus DNA and p16INK4a positivity in vulvar cancer and vulvar intraepithelial neoplasia: a systematic review and meta-analysis. *The Lancet Oncology*, 24(4), pp.403-414.
9. Hanahan, D. and Weinberg, R.A., 2000. The hallmarks of cancer. *cell*, 100(1), pp.57-70.
10. Zhang, J., Yang, P.L. and Gray, N.S., 2009. Targeting cancer with small molecule kinase inhibitors. *Nature reviews cancer*, 9(1), pp.28-39.
11. Vainonen, J.P., Momeny, M. and Westermarck, J., 2021. Druggable cancer phosphatases. *Science translational medicine*, 13(588), p.eabe2967.
12. Brivanlou, A.H. and Darnell Jr, J.E., 2002. Signal transduction and the control of gene expression. *Science*, 295(5556), pp.813-818.
13. Sever, R. and Brugge, J.S., 2015. Signal transduction in cancer. *Cold Spring Harbor perspectives in medicine*, 5(4), p.a006098.
14. Hoffmann, K., Berger, H., Kulbe, H., Thillainadarasan, S., Mollenkopf, H.J., Zemojtel, T., Taube, E., Darb-Esfahani, S., Mangler, M., Sehouli, J. and Chekerov, R., 2020. Stable expansion of high-grade serous ovarian cancer organoids requires a low-Wnt environment. *The EMBO journal*, 39(6), p.e104013.
15. Zhang, W., Klinkebiel, D., Barger, C.J., Pandey, S., Guda, C., Miller, A., Akers, S.N., Odunsi, K. and Karpf, A.R., 2020. Global DNA hypomethylation in epithelial ovarian cancer: passive demethylation and association with genomic instability. *Cancers*, 12(3), p.764.
16. Kirschner, M.W., 2005. The meaning of systems biology. *Cell*, 121(4), pp.503-504.
17. Gehlenborg, N., O'donoghue, S.I., Baliga, N.S., Goesmann, A., Hibbs, M.A., Kitano, H., Kohlbacher, O., Neuweger, H., Schneider, R., Tenenbaum, D. and Gavin, A.C., 2010. Visualization of omics data for systems biology. *Nature methods*, 7(Suppl 3), pp.S56-S68.
18. Fitzgerald, J.B., Schoeberl, B., Nielsen, U.B. and Sorger, P.K., 2006. Systems biology and combination therapy in the quest for clinical efficacy. *Nature chemical biology*, 2(9), pp.458-466.
19. Butcher, E.C., Berg, E.L. and Kunkel, E.J., 2004. Systems biology in drug discovery. *Nature biotechnology*, 22(10), pp.1253-1259.
20. Dey, S.S., Kester, L., Spanjaard, B., Bienko, M. and Van Oudenaarden, A., 2015. Integrated genome and transcriptome sequencing of the same cell. *Nature biotechnology*, 33(3), pp.285-289.
21. Eliyahu, D., Raz, A., Gruss, P., Givol, D. and Oren, M., 1984. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature*, 312(5995), pp.646-649.
22. Sims, A.H., Howell, A., Howell, S.J. and Clarke, R.B., 2007. Origins of breast cancer subtypes and therapeutic implications. *Nature Clinical Practice Oncology*, 4(9), pp.516-525.

- 1           23. Chaffer, C.L. and Weinberg, R.A., 2011. A perspective on cancer cell metastasis. *science*,  
2 331(6024), pp.1559-1564.
- 3           24. Reya, T., Morrison, S.J., Clarke, M.F. and Weissman, I.L., 2001. Stem cells, cancer, and cancer  
4 stem cells. *nature*, 414(6859), pp.105-111.
- 5           25. Battle, E. and Clevers, H., 2017. Cancer stem cells revisited. *Nature medicine*, 23(10),  
6 pp.1124-1134.
- 7           26. Kreso, A. and Dick, J.E., 2014. Evolution of the cancer stem cell model. *Cell stem cell*, 14(3),  
8 pp.275-291.
- 9           27. Koike-Yusa, H., Li, Y., Tan, E.P., Velasco-Herrera, M.D.C. and Yusa, K., 2014. Genome-wide  
10 recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library. *Nature*  
11 *biotechnology*, 32(3), pp.267-273.
- 12           28. Shah, A.N., Davey, C.F., Whitebitch, A.C., Miller, A.C. and Moens, C.B., 2015. Rapid reverse  
13 genetic screening using CRISPR in zebrafish. *Nature methods*, 12(6), pp.535-540.
- 14           29. Mamoor, Shahan. 2024. Therapeutic targeting of catalytically available solid tumor vulnerabilities  
15 in human cancer.
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## Methods

We utilized GSE124766 for this tumor transcriptome study, measuring whole transcription in primary tumors from humans with ovarian cancer, as compared to normal ovarian (along with GSE146556 for target validation) using microarray data (published) and R-based computational methods (GEO2R).