Biomarker Testing: Which biomarker and when?

ADCs Toxicities: Optimizing Patient Management and Outcomes
Vancouver British Columbia Canada

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A GOG Foundation, Inc. Educational Program

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Disclosure – Angeles Alvarez Secord

- Financial relationships with ACCME defined ineligible companies to report over the past 24 months:

Company name	*Honoraria/ expenses*	*Consulting/ advisory board*	*Funded research*	*Royalties/ patent*	*Stock options*	*Other (please specify)*
AbbVie, Aravive, AstraZeneca, Clovis, Eisai, Ellipses Pharma, GSK, I-MAB Biopharma, Immunogen, Merck, Oncoquest, Roche/ Genentech, Seagen, Inc, Theradex, VBL, NRG Oncology, National Cancer Trial Network			X Paid to Duke			
AstraZeneca, Clovis, GSK, Immunogen, Imvax, Merck, Mersana, Natera, Onconova, Oncoquest		X (Uncompensated)				
Aravive, Roche/Genentech, VBL, and Oncoquest						X - Clinical trial Steering Committees (Uncompensated)
@Point of Care, ASCO, Clinical Care Options, CurioScience, PeerView, Bio ASCEND, Research to Practice	X					
GOG Foundation, NRG Oncology, SGO, AAOGF						X- (travel/accommodations/ expenses)

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Approved ADCs for Solid Tumors

Generic Name	FDA Approval	Payload	Mechanism of Action	Target	Cancer Indication
Trastuzumab emtansine	2013	Maytansinoid (DM1)	Anti-microtubule	HER2	HER2+ Breast
Trastuzumab deruxtecan	2019	Deruxtecan (Dxd)	Topoisomerase I inhibitor	HER2	HER2+ Breast; HER2-low (IHC 1+ or IHC 2+/ISH-) Breast; NSCLC with ERBB2 mutations; HER2+ gastric or gastroesophageal junction adenocarcinoma
Enfortumab vedotin	2019	MMAE	Anti-microtubule	Nectin-4	Urothelial*
Sacituzumab govitecan	2020	SN-38	Topoisomerase I inhibitor	Trop-2	Breast and Urothelial*
Tisotumab vedotin	2021	MMAE	Anti-microtubule	Tissue Factor	Cervix*
Mirvetuximab soravtansine	2022	Maytansinoid (DM4)	Anti-microtubule	Folate Receptor α	Ovary

MMAE: Monomethyl auristatin E; **NSCLC: non-small cell lung cancer; SN-38, active metabolite of irinotecan**

Tisotumab Vedotin: Targeting Tissue Factor

- TF is a transmembrane protein that initiates coagulation and involved in angiogenesis, cell adhesion, motility, and cell survival
- TF can be determined in tumor tissue by IHC, also in blood and urine.
- Aberrantly expressed in numerous solid tumors and can be associated with poor prognosis.
  - Glioma, breast, lung, colon, bladder, prostate, pancreatic, ovarian and hepatocellular carcinoma in tumor cells and/or tumor stroma and/or endothelial cells.
    - Ovarian cancer – 40-96%
    - Endometrial cancer - 50% serous subtype
    - Cervical cancer 34% - 100%
- Diagnostic and prognostic value uncertain

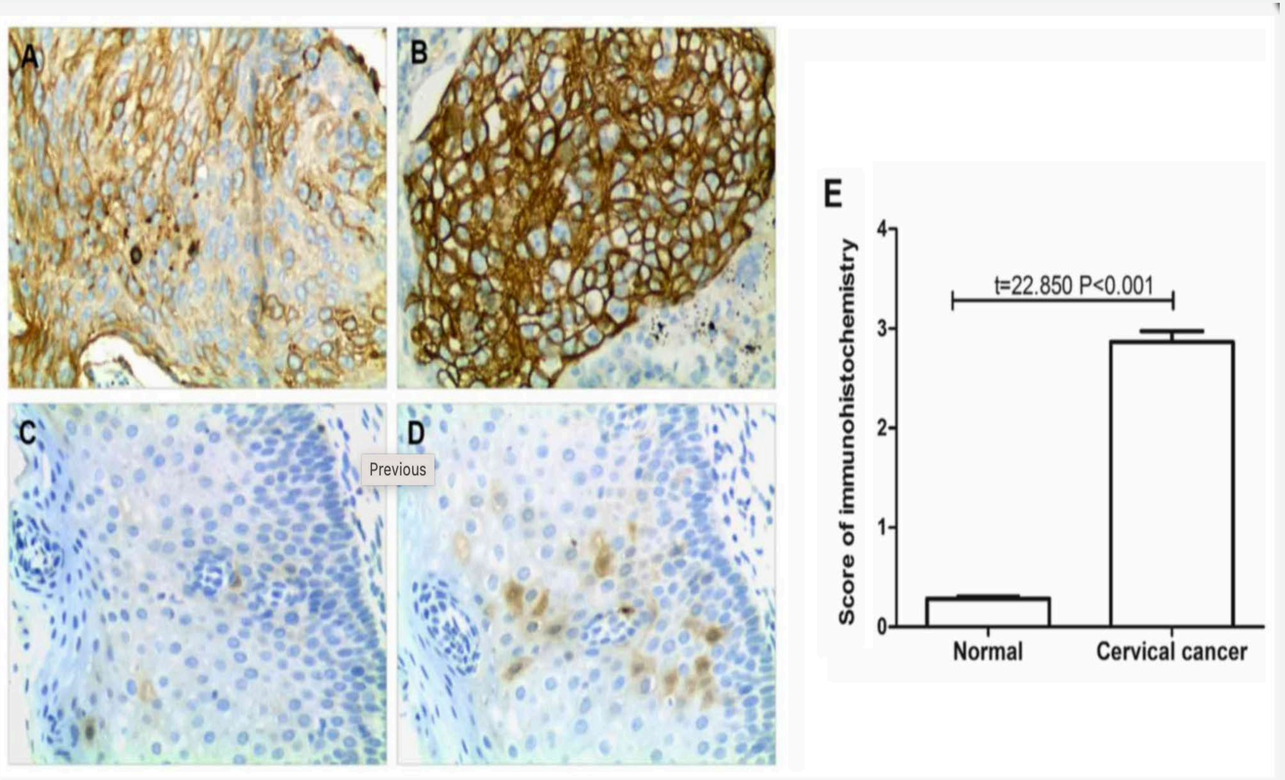
Indication	Characteristics	TF Expression (N)
Ovarian	Epithelial/Fallopian tube	40% (48)
Bladder	TCC	45% (60)
NSCLC	Squamous cell CA Non-squamous cell CA	69% (161) High percentage of TF in squamous cell CA
Pancreatic cancer	Adenocarcinoma	75% (60)
Head and Neck Cancer	Squamous cell CA	84% (55) High percentage of TF in squamous cell CA
Colon Cancer	Adenocarcinoma	72% (72)

Treatment is not based on the BIOMARKER

Hong DS CCR 2020; Chu AJ. Int J Inflam. 2011; Förster Y et al. Clin Chim Acta. 2006; Cocco E et al. BMC Cancer. 2011

Targeting Tissue Factor Staining

- Not a companion or complementary biomarker
- Biomarker scoring:
  - The % of positive cells ≤25%, 25–75% or ≥75%, were scored 0, 1 and 2
  - Then cells were scored based on color - colorless, light yellow, brown and tan, and scored 0, 1, 2 and 3
  - Then multiply TF positive cell rate score with the staining intensity score.
  - A TF- IHC score of ≥4 represented high expression and <4 represented low expression.



Treatment is not based on the BIOMARKER

Zhao et al. Experimental and Therapeutic Medicine 2018

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ADCs Under Evaluation in Gynecologic Cancers

Generic Name	Payload	Mechanism of Action	Target and Function
Tisotumab vedotin	MMAE	Anti-microtubule	Tissue Factor: involved in coagulation, angiogenesis, cell adhesion, motility, and cell survival
XB002			
Mirvetuximab soravtansine	Maytansinoid (DM4)	Anti-microtubule	Folate Receptor Alpha: Transmembrane protein. Folate transport into cells needed for metabolism, DNA synthesis, repair, and proliferation
STRO-002	Cytotoxin 3-aminophenyl hemiasterlin (SC209)		
Upifitamab Rilsodotin	Auristatin F-hydroxypropylamide	Anti-microtubule	NaPi2b: Sodium-dependent phosphate transport protein
SKB264	Belotecan	Topoisomerase I inhibitor	Trophoblast antigen 2 (TROP2): promotes cancer growth, invasion & metastasis. *Stem cell biology
Sacituzumab govitecan	SN-38		
BDC-1001	Trastuzumab conjugated to toll-like receptor 7/8 agonist	Tumor-targeting Ab + immune-stimulating Ab conjugate	Human epidermal growth factor receptor 2 (HER2): when activated promotes proliferative and anti-apoptosis signals
DB1303	Trastuzumab conjugated to P1003	Tumor-targeting Ab + Topo I inhibitor	
DS-6000a	Deruxtecan	Topo I inhibitor	CDH6 (Cadherin 6): cell-cell adhesion, organ development, and epithelial-mesenchymal transition

SN-38, active metabolite of irinotecan

Mirvetuximab soravtansine: Targeting Folate Receptor Alpha

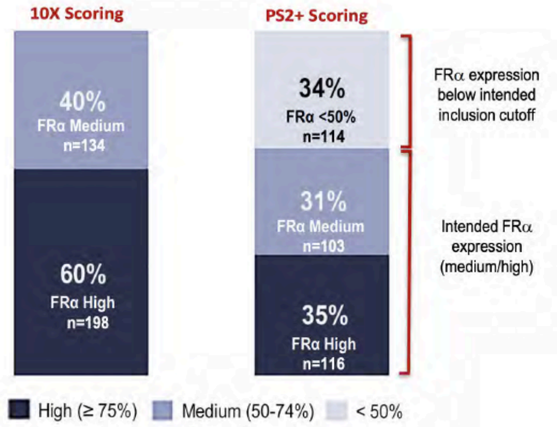
FORWARD-1

It's a Biomarker story

FORWARD I 10X SCORING COMPARED WITH EXPLORATORY PS2+ SCORING

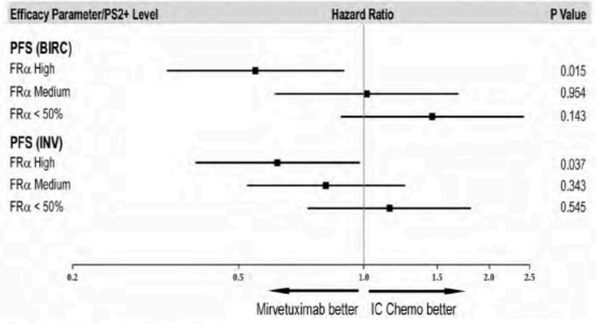
Rescoring of the FORWARD I samples using PS2+ indicates:

- 34% of patients enrolled in FORWARD I had low FRα levels that should have precluded enrollment; and
- the protocol-defined FRα high subset contained patients with a mixture of FRα expression levels

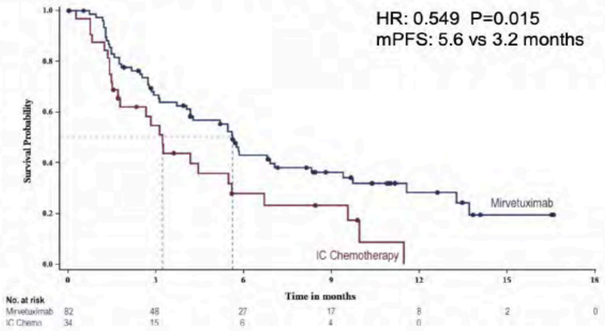


PS2+ RE-SCORING: PFS TRENDS ACROSS SUBGROUPS

PFS Hazard Ratio Plot



PFS (by BIRC) - FRα High (n=116)



P values from unstratified log-rank test

PS2+ RE-SCORING: TRENDS ACROSS SUBGROUPS

Endpoint	FRα < 50% (n=114) (Mirv vs IC Chemo)	FRα Medium (n=103) (Mirv vs IC Chemo)	FRα High (n=116) (Mirv vs IC Chemo)
PFS by BIRC (mo.)	HR: 1.458 (0.878, 2.420) mPFS: 3.8 vs 5.5	HR: 1.015 (0.611, 1.687) mPFS: 4.3 vs 5.6	HR: 0.549 (0.336, 0.897) mPFS: 5.6 vs 3.2
ORR by BIRC 95% CIs	16% vs 16% (8%, 26%) vs (6%, 31%)	28% vs 18% (18%, 40%) vs (7%, 35%)	29% vs 6% (20%, 40%) vs (1%, 20%)
OS (*August 2019*) (mo.)	HR: 0.923 (0.548, 1.554) mOS: 14.0 vs 13.4	HR: 0.936 (0.542, 1.616) mOS: 15.9 vs 20.7	HR: 0.678 (0.410, 1.119) mOS: 16.4 vs 11.4
PFS by INV (mo.)	HR: 1.149 (0.732, 1.803) mPFS: 4.0 vs 4.5	HR: 0.810 (0.523, 1.254) mPFS: 5.1 vs 2.8	HR: 0.619 (0.394, 0.975) mPFS: 5.6 vs 3.7
ORR by INV 95% CIs	18% vs 21% (11%, 29%) vs (10%, 37%)	36% vs 24% (25%, 49%) vs (11%, 41%)	38% vs 9% (27%, 49%) vs (2%, 24%)

Moore, K, ESMO 2019

Mirvetuximab soravtansine: Targeting Folate Receptor Alpha

- “On November 14, 2022, the Food and Drug Administration granted accelerated approval to mirvetuximab soravtansine-gynx for adult patients with folate receptor alpha (FRα) positive, platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer. . . Patients are selected for therapy based on an FDA-approved test. . . . the FDA also approved the . . .FOLR1 (FOLR-2.1) RxDx Assay . . . as a companion diagnostic device to select patients for the above indication.”

- Initially FRα testing available through four labs

  - Caris, Labcorp, LMC Pathology Services, and Neogenomics.

- At Duke in-house testing will eventually be possible - but requires special equipment and validation through Roche.

Targeting FRα: **More about the** Companion Diagnostic

Folate receptor 1 protein (FOLR1) = folate receptor alpha (FRα)

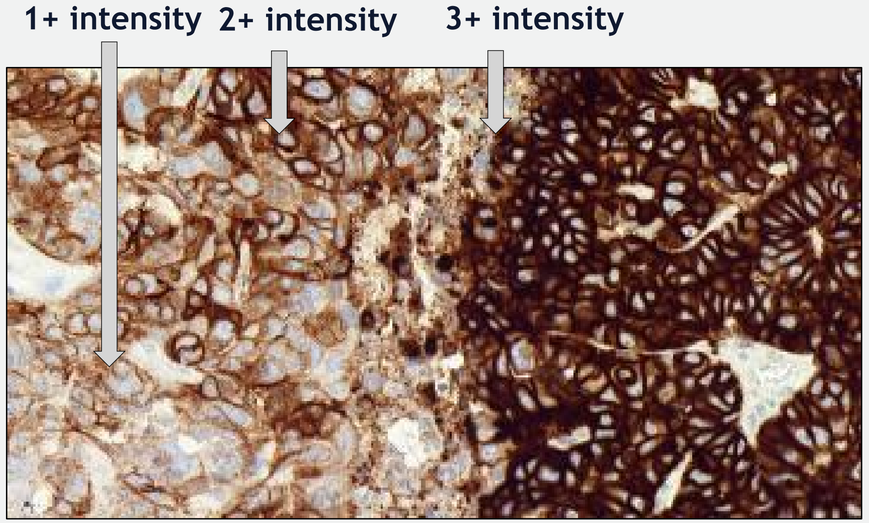
FOLR1 staining is performed utilizing the Ventana FOLR1 FDA approved protocol using the BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit and ancillary reagents.

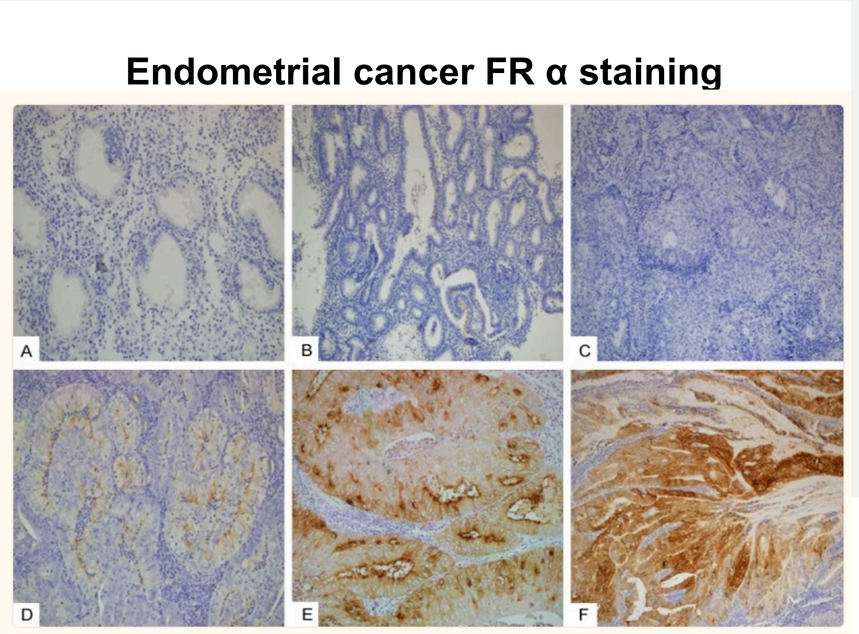
A minimum of 100 viable neoplastic cells is recommended for FOLR1 testing. FOLR1 protein expression is defined as: tumor cells showing 2+ and/or 3+ membrane staining. The specimen should be considered to be POSITIVE for FLOR1 expression if >=75 of viable tumor cells show 2+ and/or 3+ staining. The specimen is considered NEGATIVE for FLOR1 expression if <75% of viable tumor cells show 2+ and/or 3+ staining.

Cases with 2+ and/or 3+ membrane staining for FOLR1 in 65-85% of tumor cells may be reviewed by additional pathologist(s) to determine consensus scoring.

Targeting FRα : More about the Companion Diagnostic

- FRα is a cell surface folate receptor which mediates folate transport into epithelial cells
- FRα expression is limited on normal cells, but is upregulated on cancers, primarily ovarian, but also endometrial, and triple negative breast cancers.
  - High levels defined as ≥75% tumor cells staining with 2+ intensity.
  - Expressed at some level in ~90% of ovarian carcinomas
    - ~ 35-40% of ovarian cancer patients express high levels of FRα
  - 50.5% in endometrial cancers
  - 25% in cervical cancers differed according to histology (SCC vs. non-SCC, 14.9% vs. 37.5%)



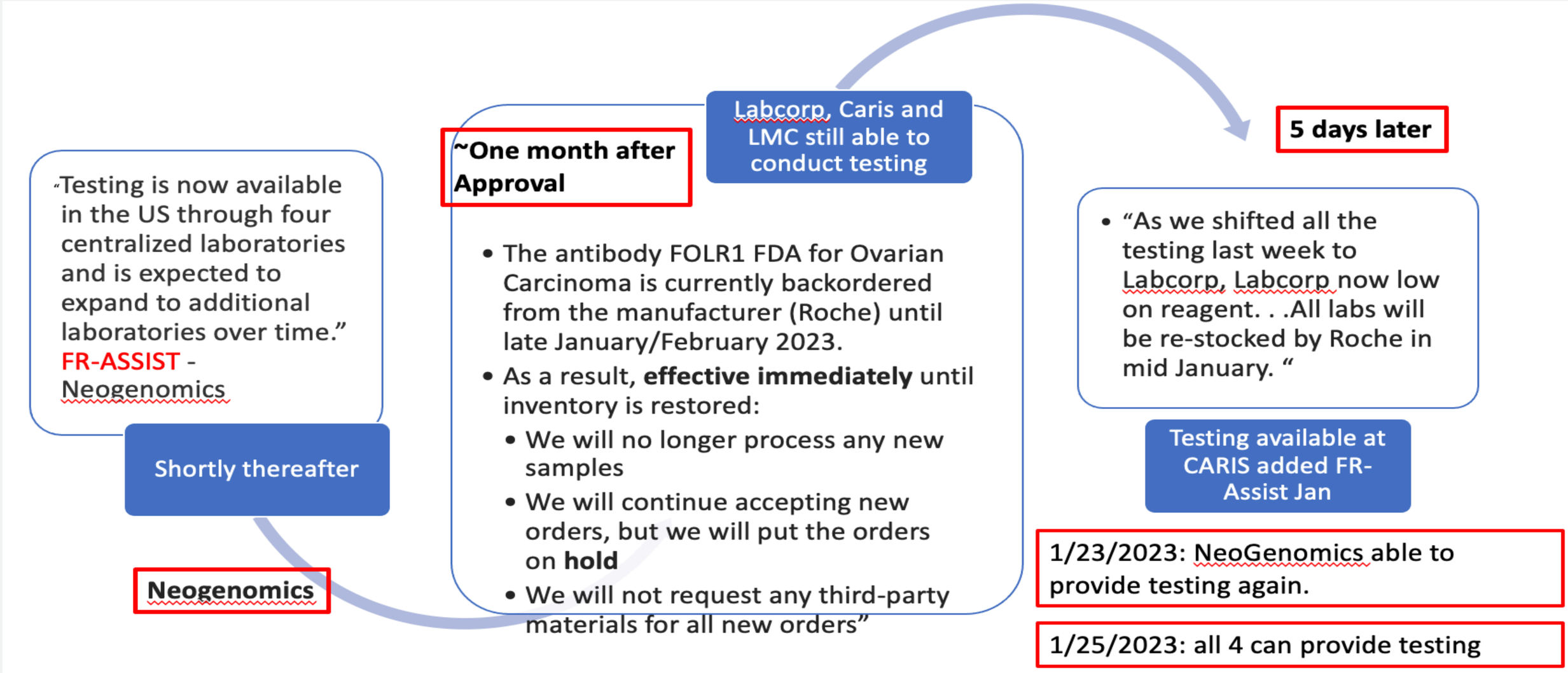


Senol et al. Int J Clin Exp Pathol 2015; Yazaki S et al. J Gynecol Oncol 2022

Targeting FRα: **More about the** Companion Diagnostic

- Testing can be done on fresh or archival tissue
  - When?
    - Start testing patients at diagnosis?
    - Versus recurrence?
  - Testing newly diagnosed patients will determine treatment options at the time of progression to platinum resistance.
  - Cost-effectiveness?

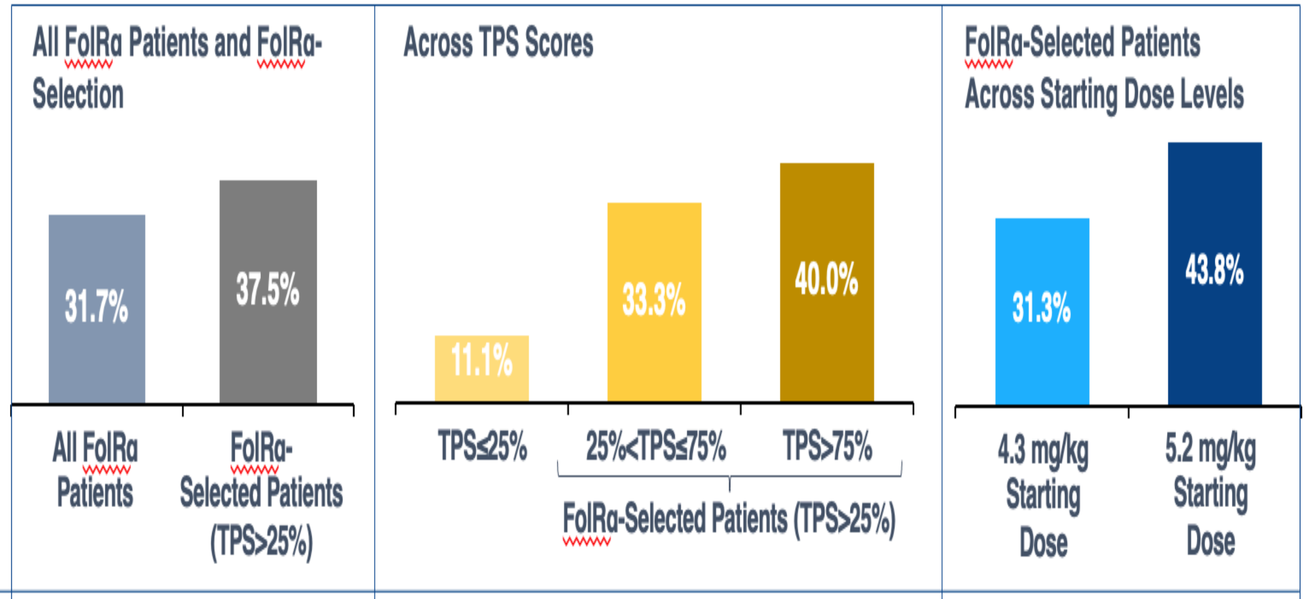
Timeline regarding testing availability



STRO-002: Targeting Folate Receptor Alpha

- Ventana FOLR1 testing
- ORR 31.7% in all FolRα + and 37.5% TPS>25%
- TPS >25% appears to be the threshold for anti-tumor activity
  - No scoring needed

RECIST-Evaluable ORR (%), Median DOR (%), and Median PFS

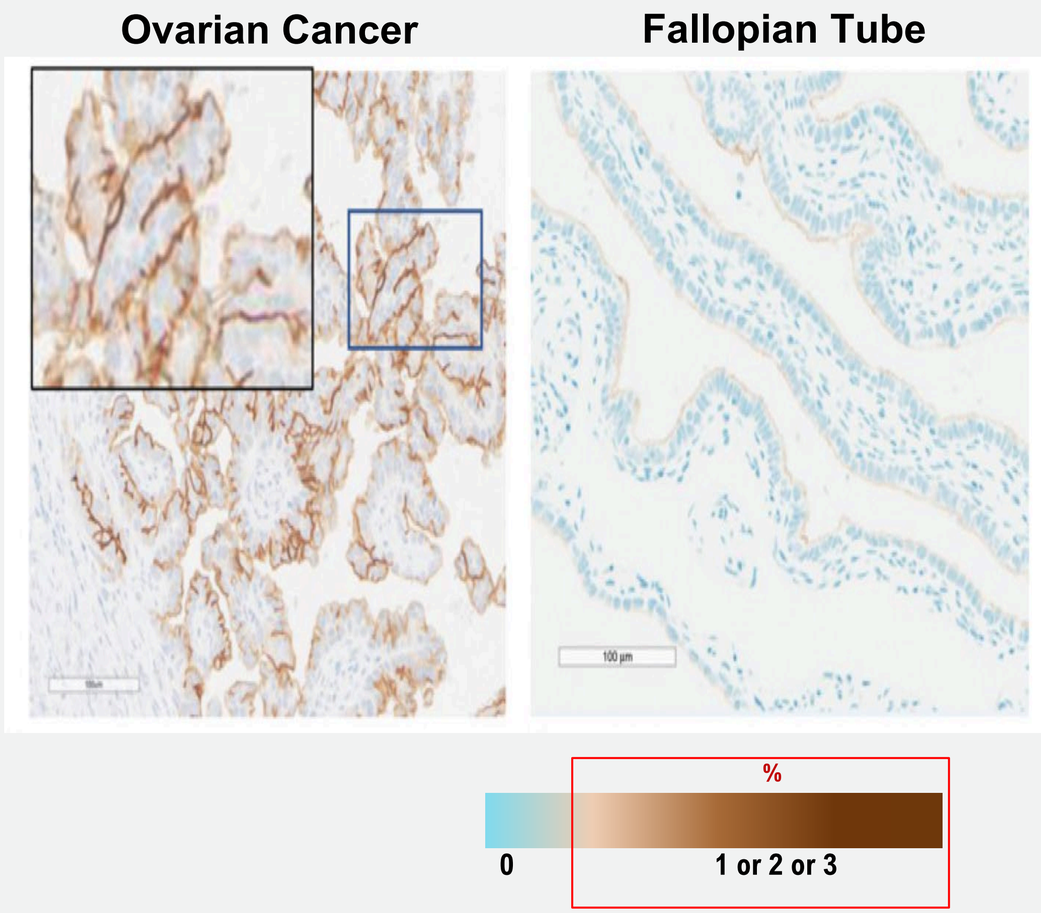


	All FolRα Patients	FolRα-Selected Patients (TPS>25%)	TPS≤25%	25%<TPS≤75%	TPS>75%	4.3 mg/kg Starting Dose	5.2 mg/kg Starting Dose
RECIST-Evaluable Patients	N=41	N=32	N=9	N=12	N=20	N=16	N=16
PR	13	12	1	4	8	5	7
ORR (95%, CI), %	31.7 (18.1, 48.1)	37.5 (21.1, 56.3)	11.1 (0.3, 48.3)	33.3 (10.0, 65.1)	40.0 (19.1, 63.9)	31.3 (11.0, 58.7)	43.8 (19.8, 70.1)
Median DOR (95% CI), mo	5.4 (2.9, 11.0)	5.5 (2.5, 11.0)	2.9	5.6 (2.5, NE)	5.5 (2.4, NE)	13 (4.5, NE)	5.4 (2.4, 6.1)
Patients for median PFS	n=44	n=35	n=9	n=12	n=23	n=19	n=16
Median PFS (95% CI), mo	4.3 (4.0, 6.3)	6.1 (4.1, 7.0)	3.8 (1.3, 4.2)	6.4 (1.4, 10.4)	5.8 (4.0, 6.6)	6.1 (4.0, 8.3)	6.6 (2.9, 7.6)

Naumann W et al. abstract presentation

Targeting NaPi2b

- NaPi2b target is encoded by *SLC34A2*) a member of the SLC34 family
- Sodium-dependent phosphate transporter broadly expressed in numerous cancers including HGSOC, tubal, peritoneal, thyroid, breast and NSCLC.
  - NaPi2b genetic knockout is embryonically lethal
  - Expression of *SLC34A2* is driven by PAX8
- NaPi2b is a lineage antigen (not an oncogene)
  - Stable expression throughout disease course

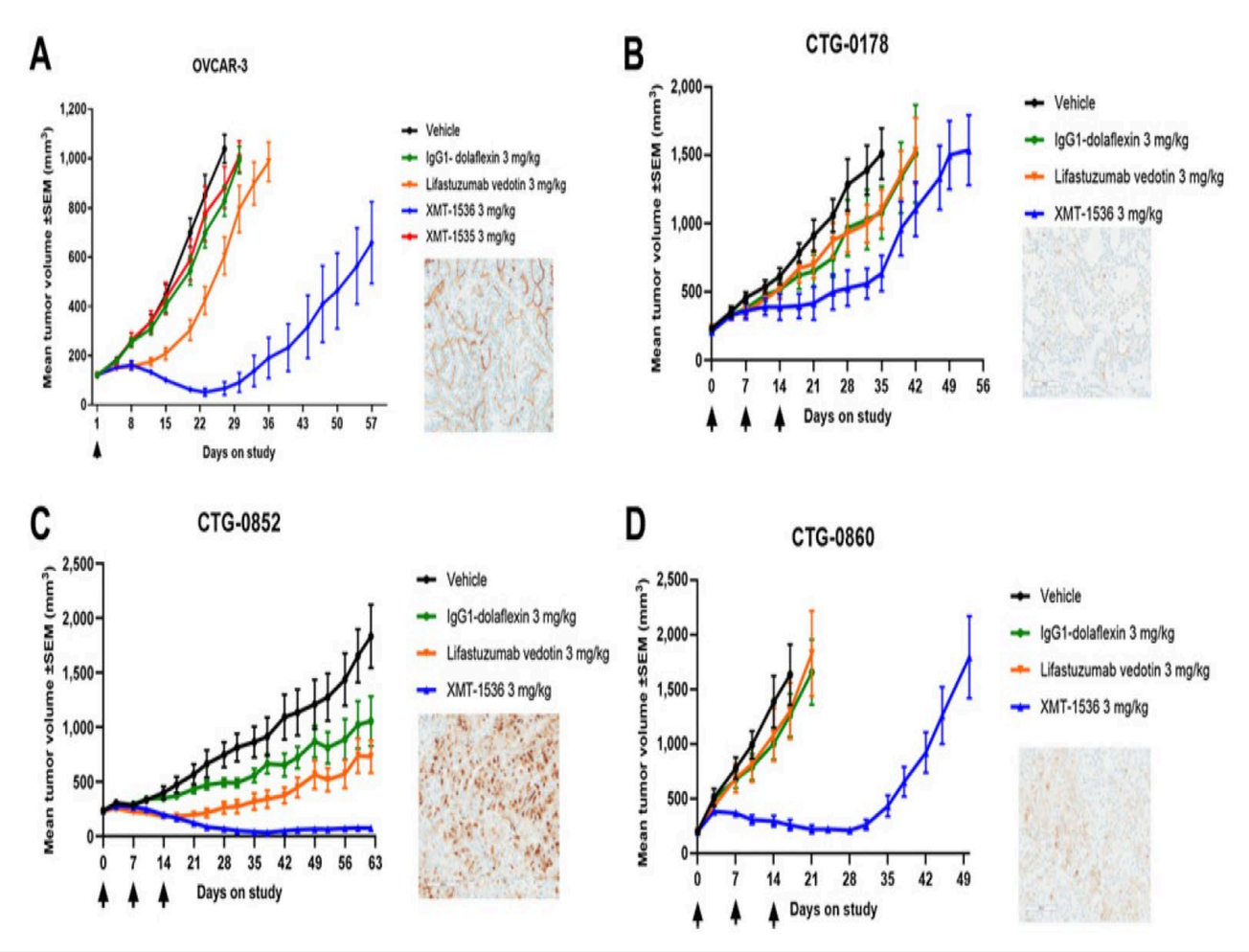


A TPS of ≥ 75 is considered to a be a positive score

Bodyak ND et al. Mol Cancer Ther. 2021;20(5):885–895; Tolcher AW et al. ASCO Annual Meeting 2019; Abstract 3010; Lin K et al. Clin Cancer Res. 2015;21(22):5139–5150.

Targeting NaPi2b

- NaPi2b in ovarian cancer
  - Initially thought to have limited expression in normal tissues.
  - Subsequent findings confirmed high expression of NaPi2b in benign fallopian tube epithelium and well-differentiated serous, endometrioid, (lower expression in mucinous ovarian carcinomas).
  - Absent in normal ovary epithelium.
  - 2/3 of HGSOC



Bodyak ND et al. Mol Cancer Ther. 2021; Banerjee et al. Cancer Treatment Reviews 2023

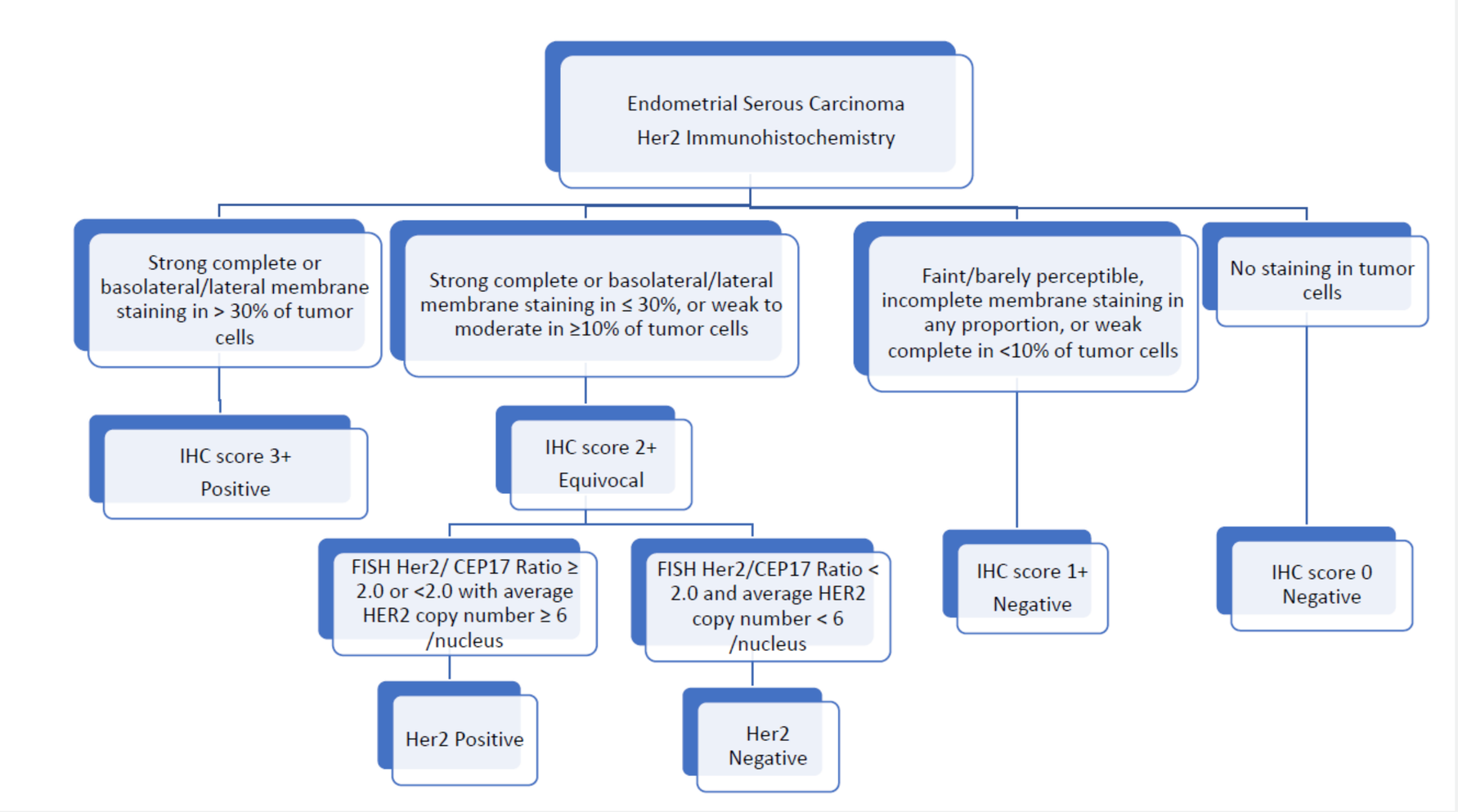
Targeting HER2 in Gynecologic Cancers

- Endometrial Cancer
  - Fader et al. trastuzumab trial; overexpressed in 30% of USC
  - Addition of trastuzumab to paclitaxel and carboplatin was endorsed by the NCCN in 2019
- Cervical cancer
  - HER2-+ staining in ~39 % of cases with stage 1B/IIA tumors
- Ovarian cancer
  - Amplification: 14%.
  - Highest in mucinous carcinomas (25%); mixed-type carcinomas (11.9%), clear cell carcinomas (4%), serous papillary carcinomas (3%), and endometrioid carcinomas (2.1%)
  - HER2 expression was associated with worse PFS and OS
  - In GOG160, a phase II trial evaluating trastuzumab in patients with recurrent or refractory ovarian cancer had ORR of 7.3 % in patients with HER2 overexpression (n=41)

HER2	Breast (ASCO/CAP 2007)	Breast (ASCO/CAP 2013; 2018*)	Gastric (ASCO/CAP 2016)	Colorectal (HERACLES trial)	UPSC (Fader et al.)
IHC 3+	>30% strong, uniform, complete	>10% circumferential, strong, complete	≥10%, strong complete or basolateral/lateral	≥50% strong, complete or basolateral/lateral	>30% strong complete or basolateral/lateral
FISH amplification	HER2/CEPT17 ratio >2.2 Patients with HER2/CEPT17 ratio 2-2.2 eligible	HER2/CEPT17 ratio ≥2.0 OR ratio <2.0 and HER2 signal ≥6.0/nucleus *(if IHC 2+ or 3+)	HER2/CEPT17 ratio ≥2.0 OR ratio <2.0 and HER2 signal ≥6.0/nucleus	HER2/CEPT17 ratio ≥2.0 in ≥50% of cells	HER2/CEPT17 ratio ≥2.0

Lassus H et al Gynecol Onc 2004; McCaughan H et al J Clin Pathol 2012; Hale RJ et al In J Gynecol Pathol 2013; Bellone S et al J Clin Pathol 2003; Ersoy E et al Gyn Path 2022

IHC and FISH HER2 characterization of endometrial cancer



- **HER2 IHC score incorporates both staining intensity and % of tumor cell staining**
- **Both complete and basolateral/ lateral staining patterns count towards % staining cut-off**

Buza et al. Modern Pathology 2021

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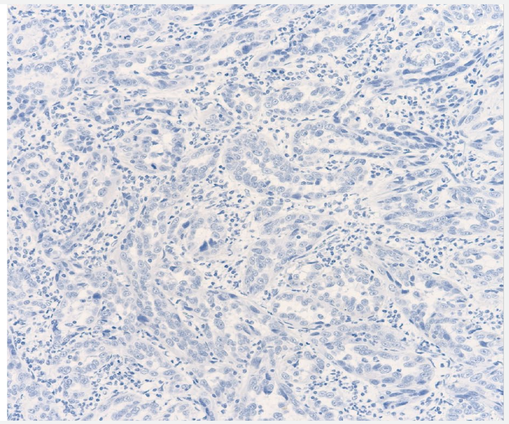
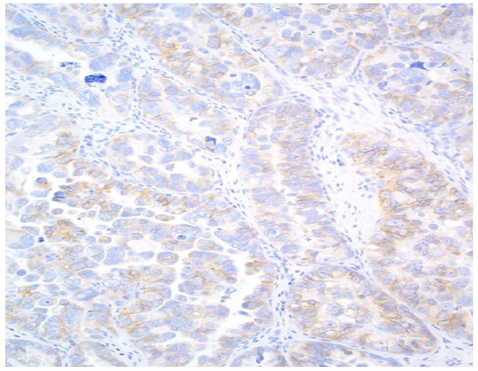
IHC characterization of protein expression in endometrial cancer

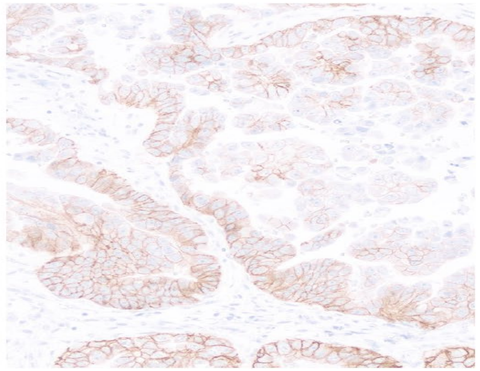
HER2 Score 0

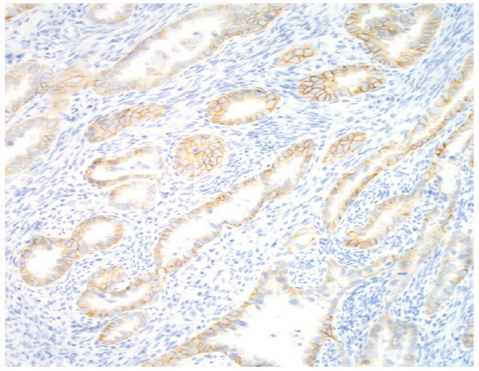
Complete absence of staining in tumor cells



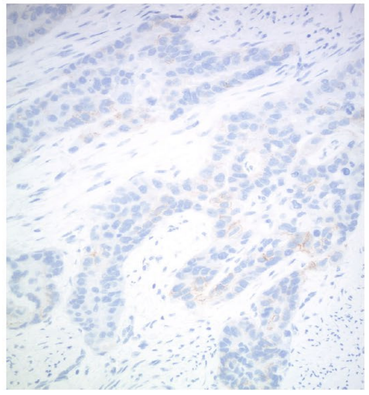
HER2 Score 2+: Weak to moderate complete or basolateral/lateral staining in ≥10% of tumor cells

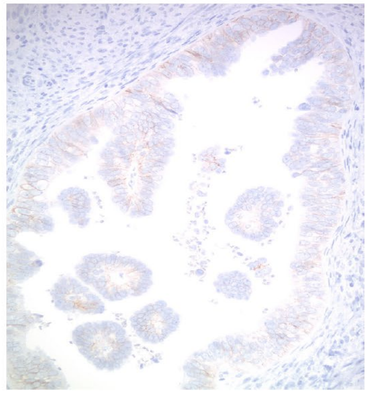




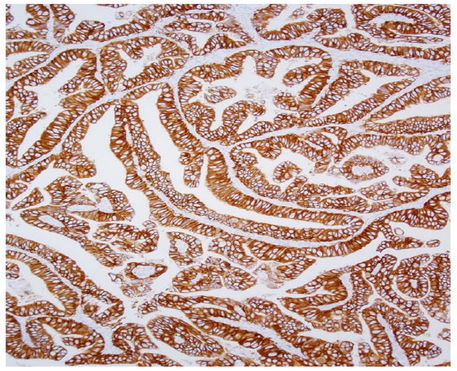


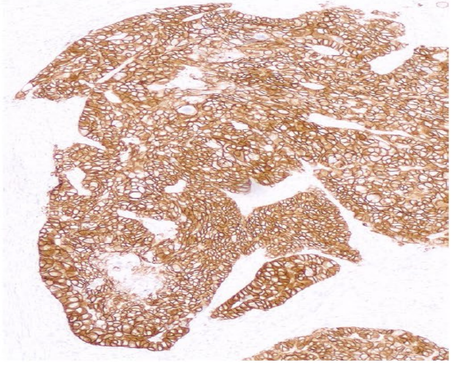
HER2 Score 1+: Faint, incomplete membrane staining in any proportion; or weak complete staining in <10% of tumor cells

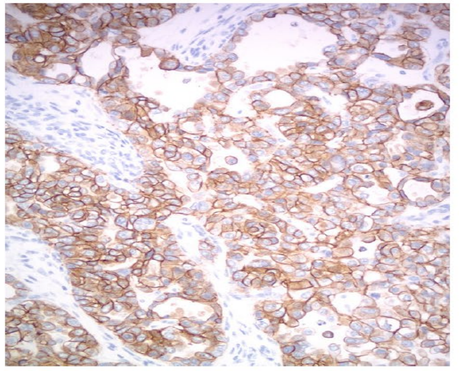




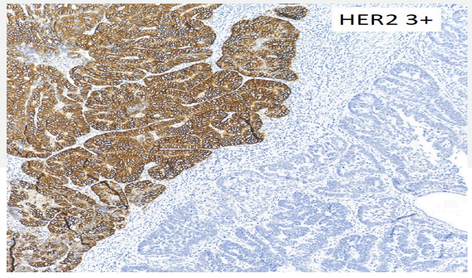
HER2 Score 3+: Strong complete or basolateral/lateral staining in > 30% of tumor cells







Intratumoral heterogeneity



Buza et al. Modern Pathology 2021

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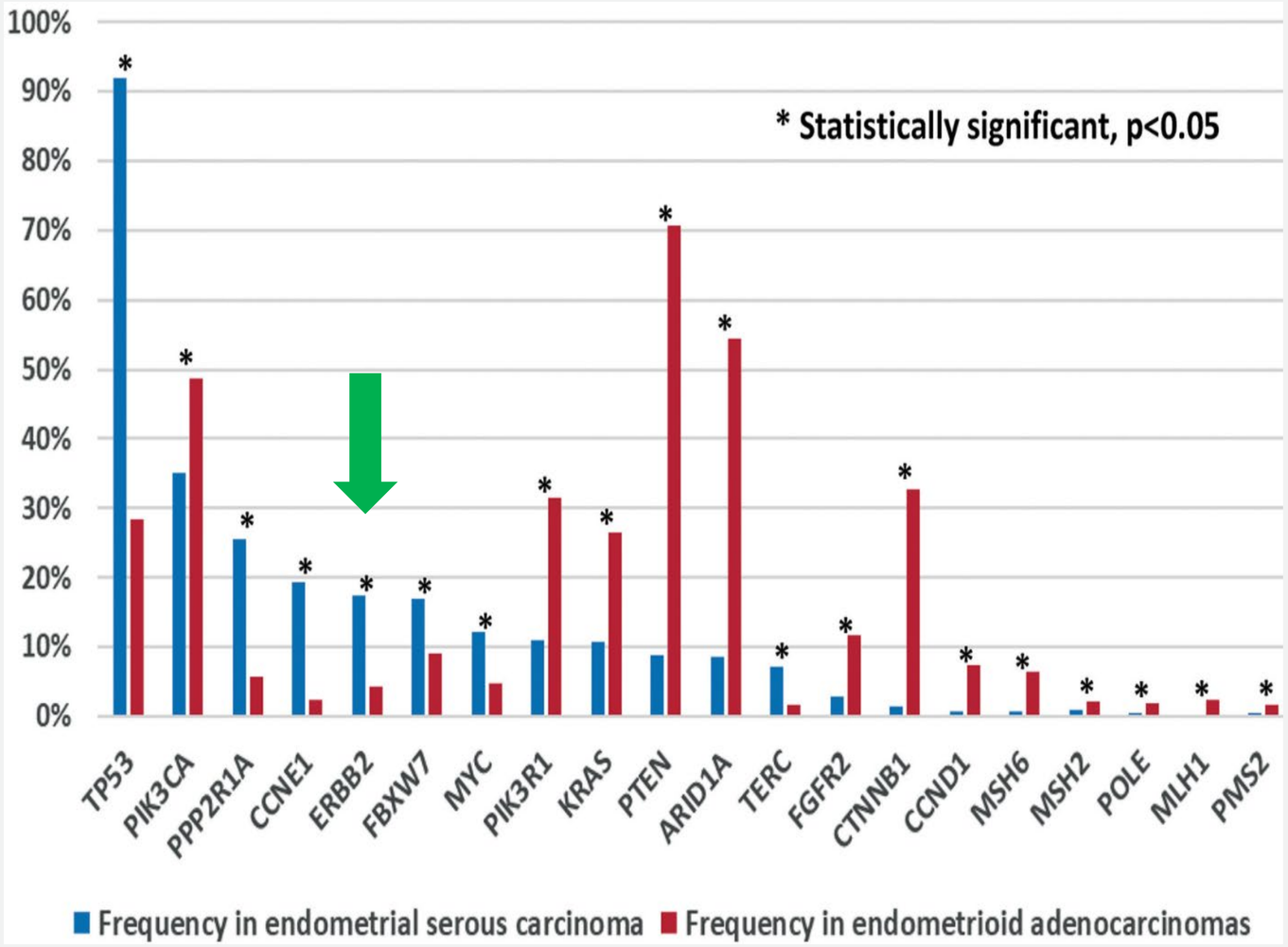
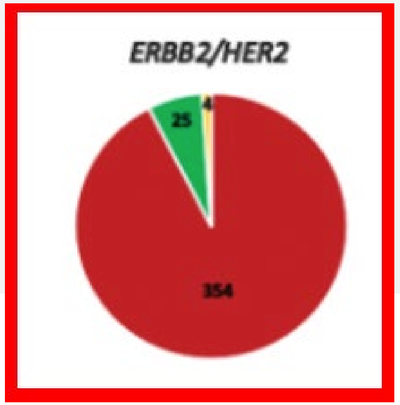
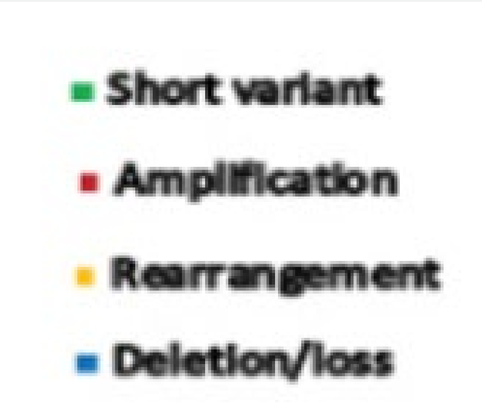
Prevalence of ERBB2/HER2 molecular aberrations



<u>Foundation Medicine Dataset</u>:
2159 UPSC (central re-review) as compared to
2346 Endometrioid (predominantly adv stg)

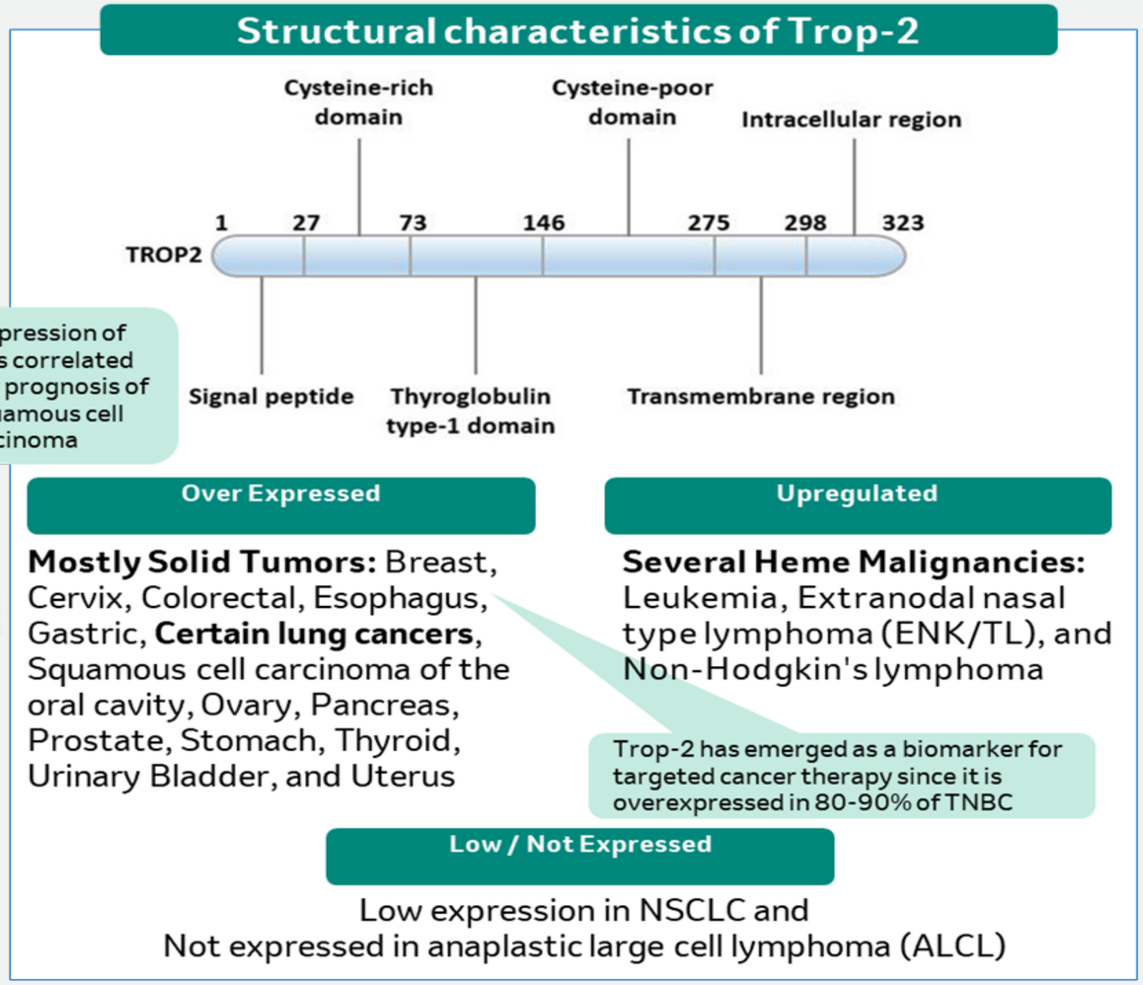
<u>UPSC</u>:
92% TP53 mutations
99% MSS, 96% TMB <10mut/MB
17% ERBB2/HER2 amplification
Overall 20% with a HER2 pathway alteration



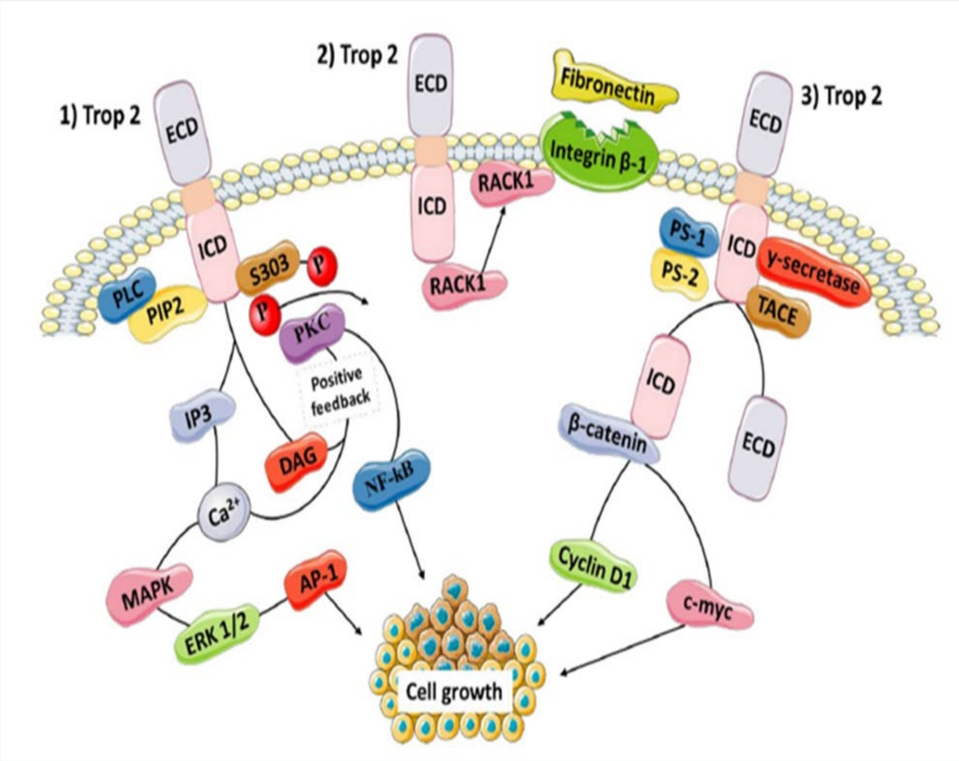


Lin et al. Gynecol Oncol 2022

Targeting TROP-2: Tumor-Associated Calcium Transducer 2



Encoded by TACSTD2 gene



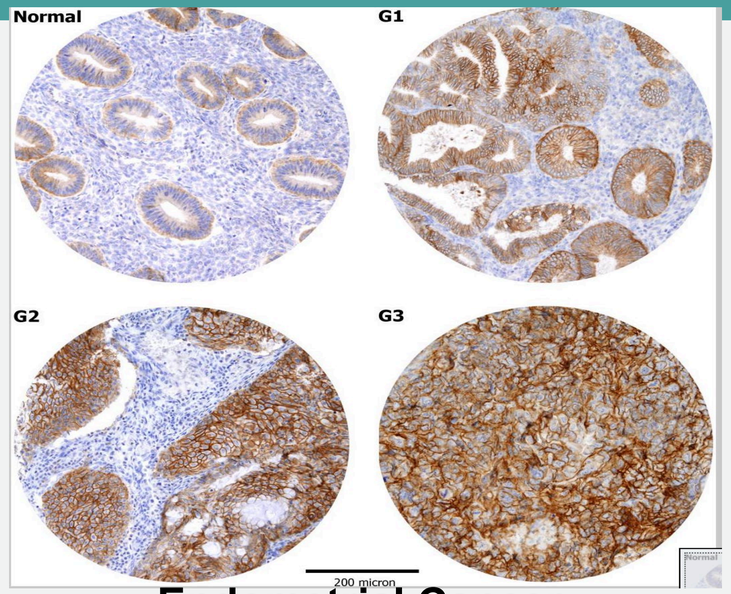
TROP-2 promotes tumor invasion and metastasis

Also considered a stem/progenitor cell marker

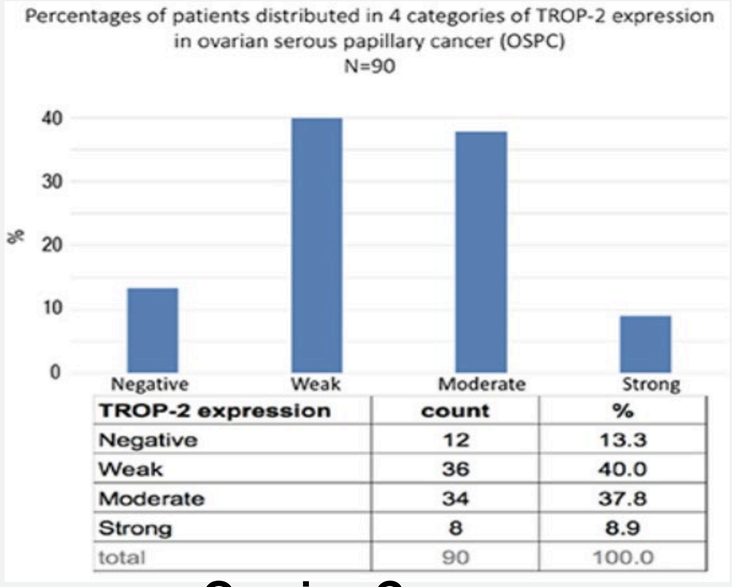
Shvartsur A et al; Tang G et al; Inamura K et al, Oncotarget, 2017 (8): 28725-35; Liao et al. Drug Development Research 2021: Lenart, S et al. Cancers 2020

Targeting TROP-2 in Gynecologic Cancers

- Endometrial cancer
  - Overexpression 96% in endometrioid endometrial cancers ; 30% (3+ expression_ carcinosarcoma; and expression in 95.1% in USC
    - EEC: G3 42%; G2 31.7%; G1, 10%
  - Cytotoxicity in Trop2+ USC cells and decreased tumor growth in Trop2+ xenografts
- Cervical cancer
  - Moderate/strong diffuse staining in 95% of SCC, and 81% of adenocarcinoma/adenosquamous cancers
  - Trop-2+ cell lines highly sensitive to sacituzumab govitecan (SG)
  - Positive correlations between TROP-2 H-score and immune markers (CD3+TILs, CD8+TILs, PD-L1 CPS).
- Ovarian cancer
  - Trop-2 overexpression 47%-82%
  - Trop-2+ cells more sensitive to SG ($p < 0.05$). SG induced significant bystander killing of Trop-2- tumor cells admixed with Trop-2+ cells.
  - Anti-tumor activity seen in TROP-2 3+chemotherapy-resistant EOC xenografts.



Endometrial Cancer



TROP-2 expression	count	%
Negative	12	13.3
Weak	36	40.0
Moderate	34	37.8
Strong	8	8.9
total	90	100.0

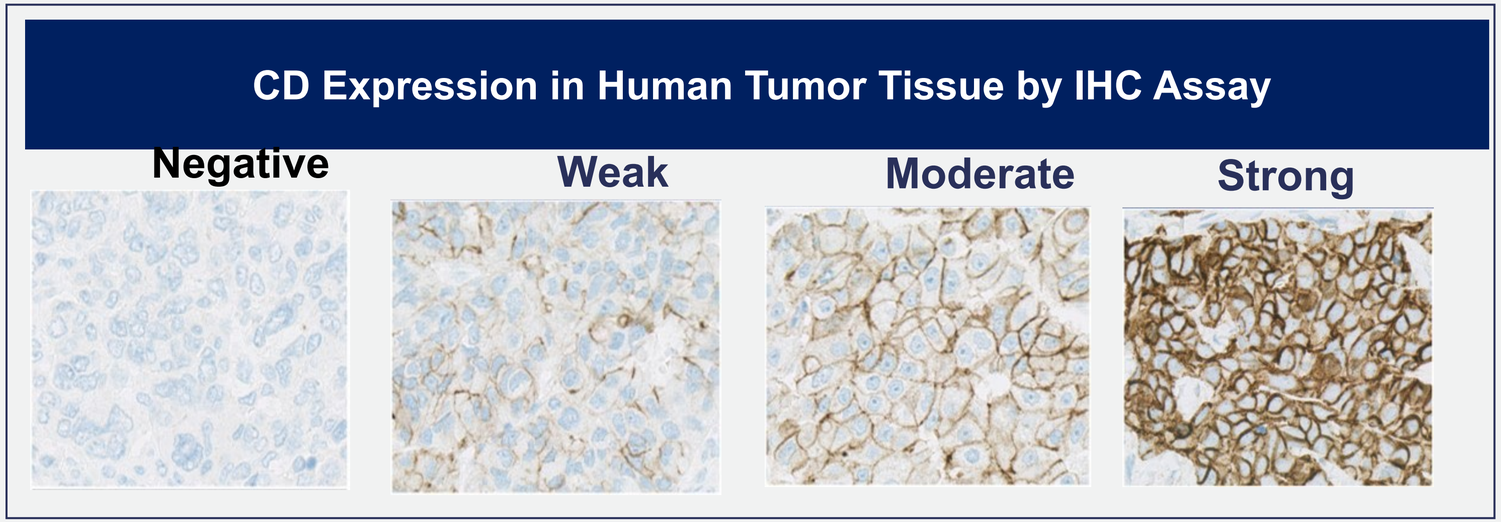
Ovarian Cancer

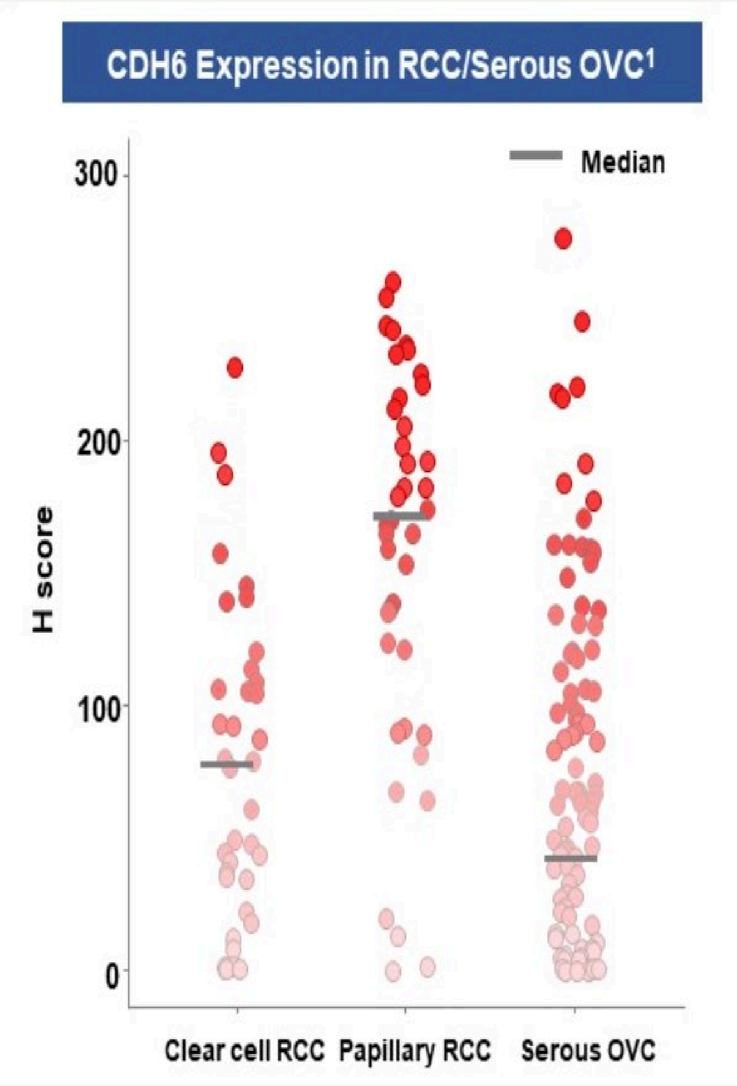
Bignotti E et al J Gynecol Cancer 2011; Han C et al. Gynecol Oncol 2020; Lopez S et al Oncotarget 2020; Zeybek B et al Sci Rep 2020; Varughese J et al. Gynecol Oncol 2011; Chiba Y et al. ESGO 2022; Perrone E et al. Front Oncol 2020;

DS-6000a: Targeting CDH6

- CDH6 is part of the cadherin family, which is involved with cell-cell adhesion, organ development, and epithelial-mesenchymal transition

- CDH6 is overexpressed in various cancers, notably in ovarian and renal cancers. Absent protein expression in endometrial and cervical cancers in TCGA dataset.

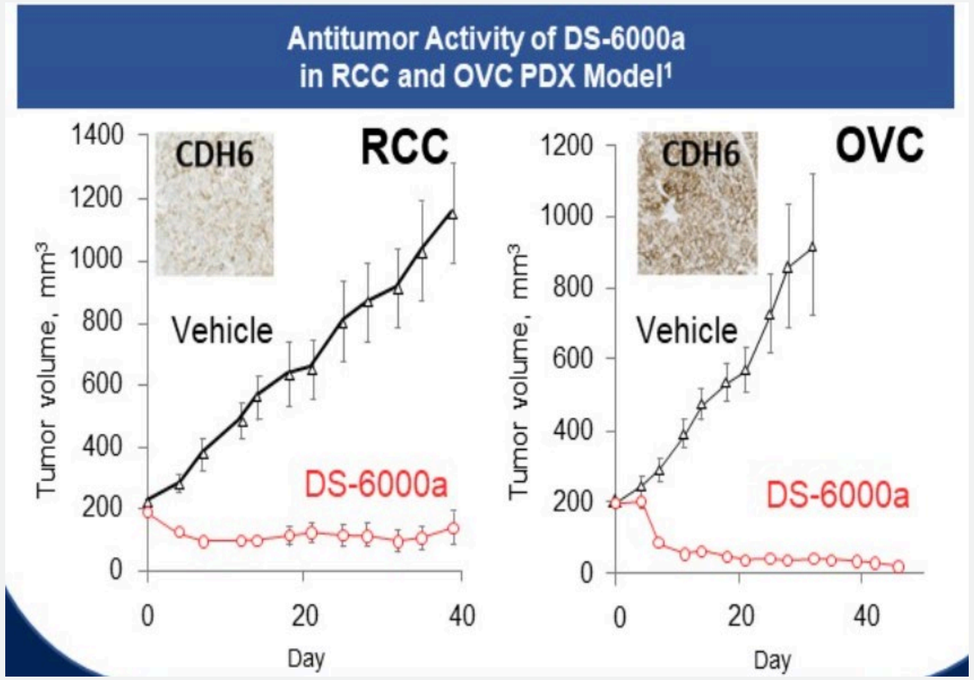




CDH6, cadherin 6; OVC, ovarian cancer; RCC, renal cell carcinoma.

Hirokazu S, et al. ESMO 2021. Abstract 10P. Hamilton E. et al. JCO 2022; https://www.proteinatlas.org/ENSG00000113361-CDH6/pathology

Targeting CDH6 Expression in Renal cell cancer and ovarian cancer



In preclinical studies DS-6000a significantly reduced tumor growth in CDH6-expressing PDX models

Hamilton E. et al. JCO 2022

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Future Directions

- Identify most efficient and cost-effective testing options
- Determine overlap of biomarker expression
- Use biomarkers to determine potential anti-neoplastic drug combinations
- Clinical trial design development

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