Development and Application of An Immunohistochemistry-based Clinical Assay for Evaluating Folate Receptor Alpha (FRα) Expression in the Clinical Setting

Abstract 3400A

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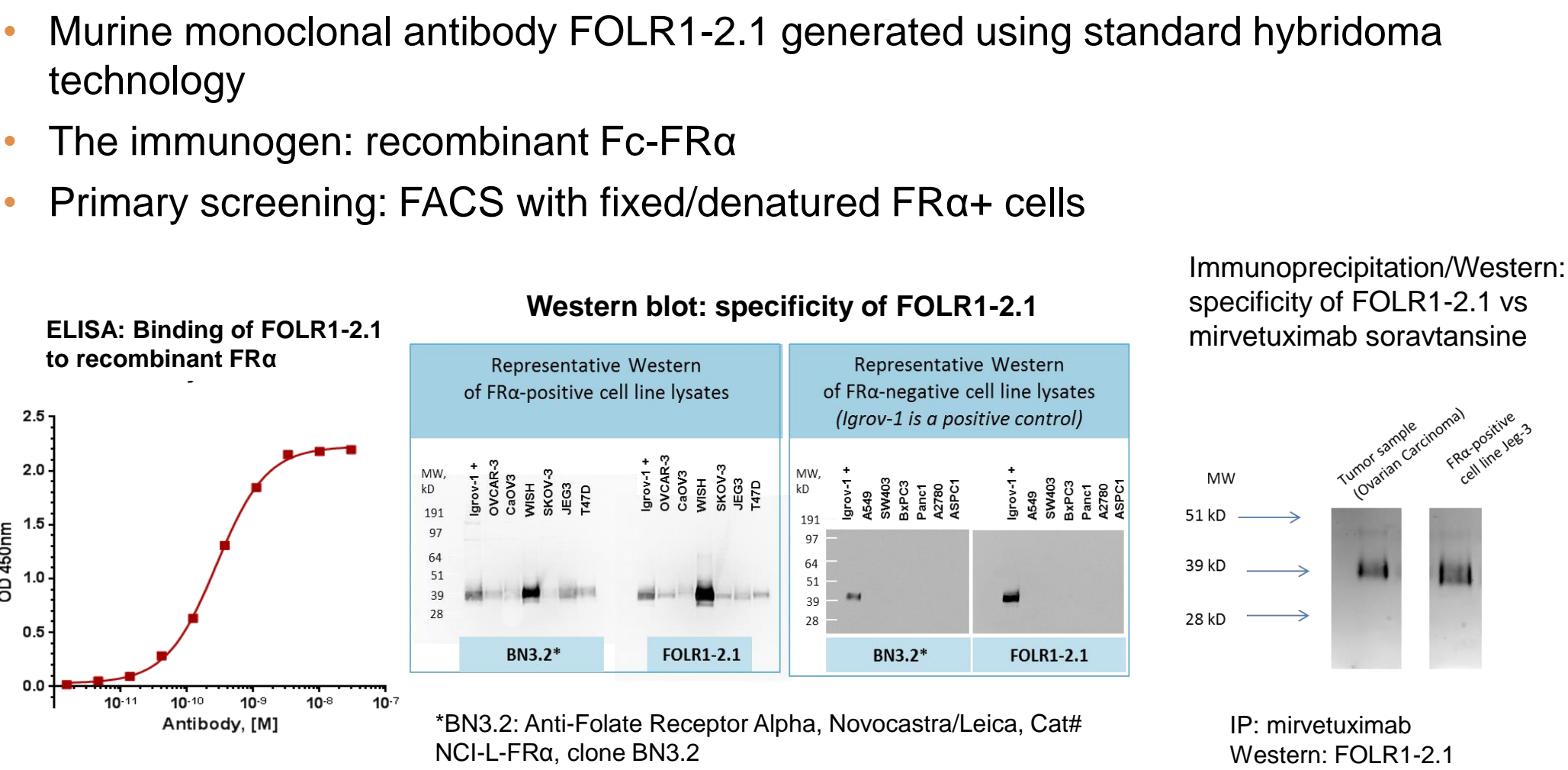
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INTRODUCTION

A biomarker-based patient selection strategy, coupled with the co-development of a companion diagnostic, is key to the successful development of molecularly targeted cancer therapeutics.
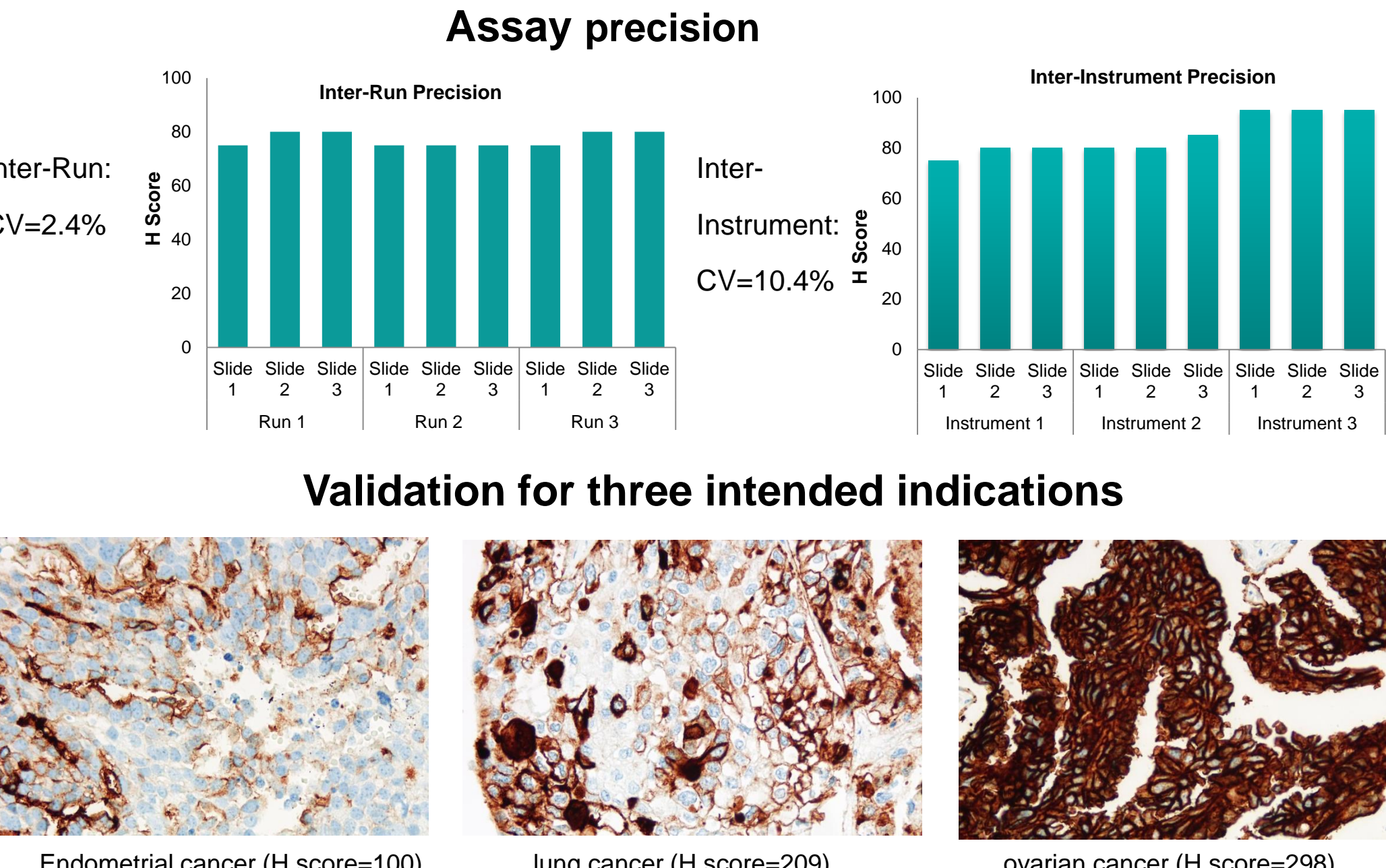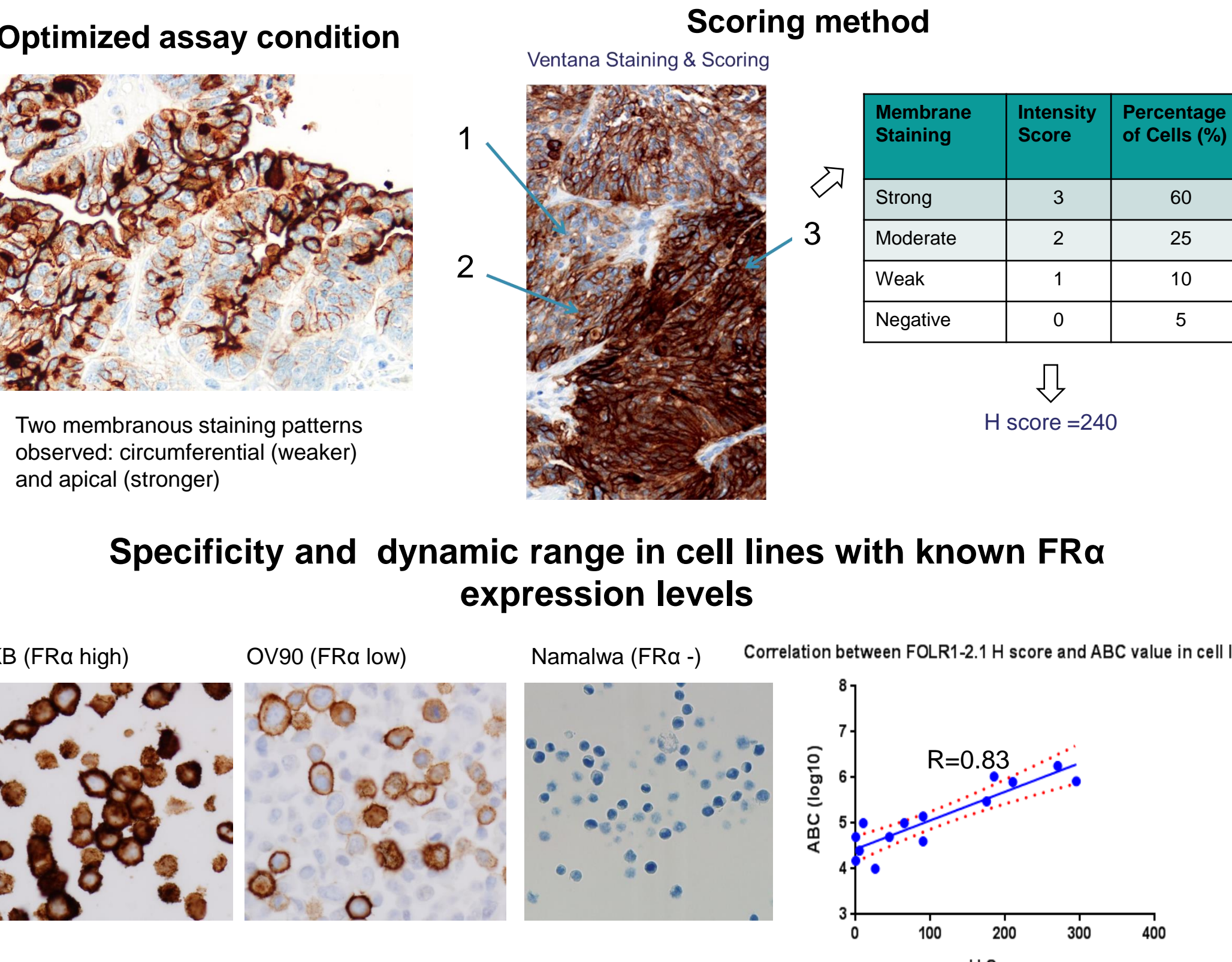
FRα is a glycosyl-phosphatidylinositol-linked membrane protein. Expression of FRα is rare in normal tissue, but frequently elevated in several solid tumor types including epithelial ovarian cancer (EOC), endometrial cancer and lung adenocarcinoma. Mirvetuximab soravtansine (IMGN853) is an antibody-drug conjugate (ADC) consisting of an anti-FRα antibody linked to DM4, a highly cytotoxic maytansinoid, *via* a cleavable disulfide linker. Preliminary evidence of clinical activity has been observed in patients receiving mirvetuximab soravtansine treatment in the first-in-human phase I clinical trial.

In this study, we report the development of an IHC-based FRα clinical-trial assay to support patient selection for mirvetuximab soravtansine. To this end, an IHC compatible murine monoclonal antibody with high specificity for human FRα was generated (Clone FOLR1-2.1). The antibody was used to develop and optimize an IHC assay that covers a broad dynamic range of FRα expression, allowing discrimination among high, moderate and low levels of expression. This semi-quantitative assay has been analytically validated per CAP/CLIA guidelines and supports intended indications including ovarian cancer, endometrial cancer and NSCLC. Additional data generated using this assay demonstrated variation in levels of FRα expression between ovarian and endometrial cancer, as well as among different histological subtypes of endometrial cancer. Comparison between pairs of primary and metastatic samples from the same patients was also carried out to understand the utility as well as potential limitation of this assay. These data help us to refine the clinical development strategy as well as the strategy for patient selection.
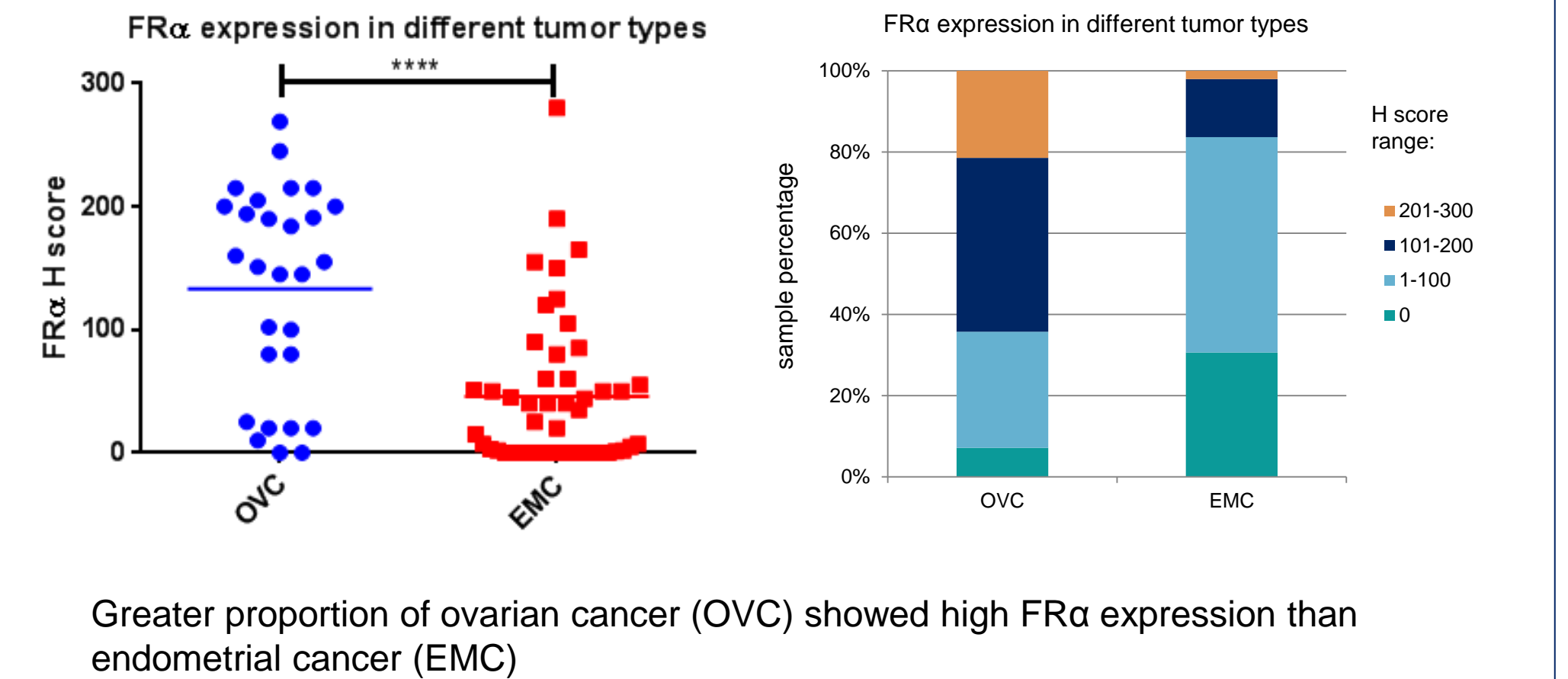
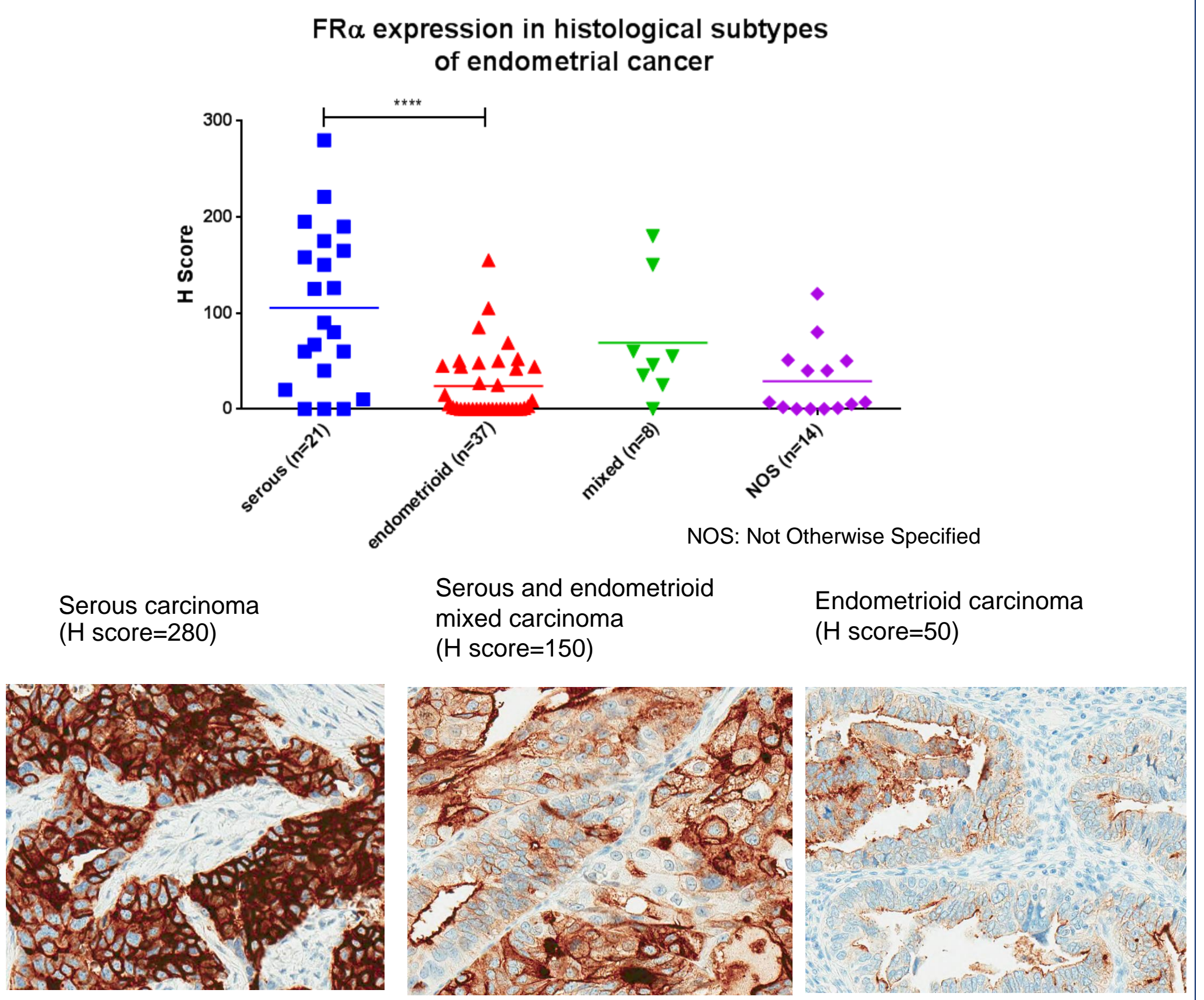## Generation of FOLR1-2.1: a FRα specific antibody for IHC

- Murine monoclonal antibody FOLR1-2.1 generated using standard hybridoma technology
- The immunogen: recombinant Fc-FRα
- Primary screening: FACS with fixed/denatured FRα+ cells

ELISA: Binding of FOLR1-2.1 to recombinant FRα

Western blot: specificity of FOLR1-2.1

Representative Western of FRα-positive cell line lysates

Representative Western of FRα-negative cell lines (Igrov-1 is a positive control)

Immunoprecipitation/Western: specificity of FOLR1-2.1 vs mirvetuximab soravtansine

IP: mirvetuximab
Western: FOLR1-2.1

*BN3.2: Anti-Folate Receptor Alpha, Novocastra/Leica, Cat# NCI-L-FRα, clone BN3.2

Development and analytical validation of a robust clinical trial assay using FOLR1-2.1

Optimized assay condition

Scoring method

Ventana Staining & Scoring

Membrane Staining	Intensity Score	Percentage of Cells (%)
Strong	3	60
Moderate	2	25
Weak	1	10
Negative	0	5

H score = 240

Two membranous staining patterns observed: circumferential (weaker) and apical (stronger)

Specificity and dynamic range in cell lines with known FRα expression levels

KB (FRα high)   OV90 (FRα low)   Namalwa (FRα -)

Correlation between FOLR1-2.1 H score and ABC value in cell lines

R=0.83

Assay precision

Inter-Run: CV=2.4%

Inter-Instrument: CV=10.4%

Validation for three intended indications

Endometrial cancer (H score=100)   lung cancer (H score=209)   ovarian cancer (H score=298)

Ovarian cancer more likely to show high FRα expression than endometrial cancer

FRα expression in different tumor types

FRα expression in different tumor types

H score range:
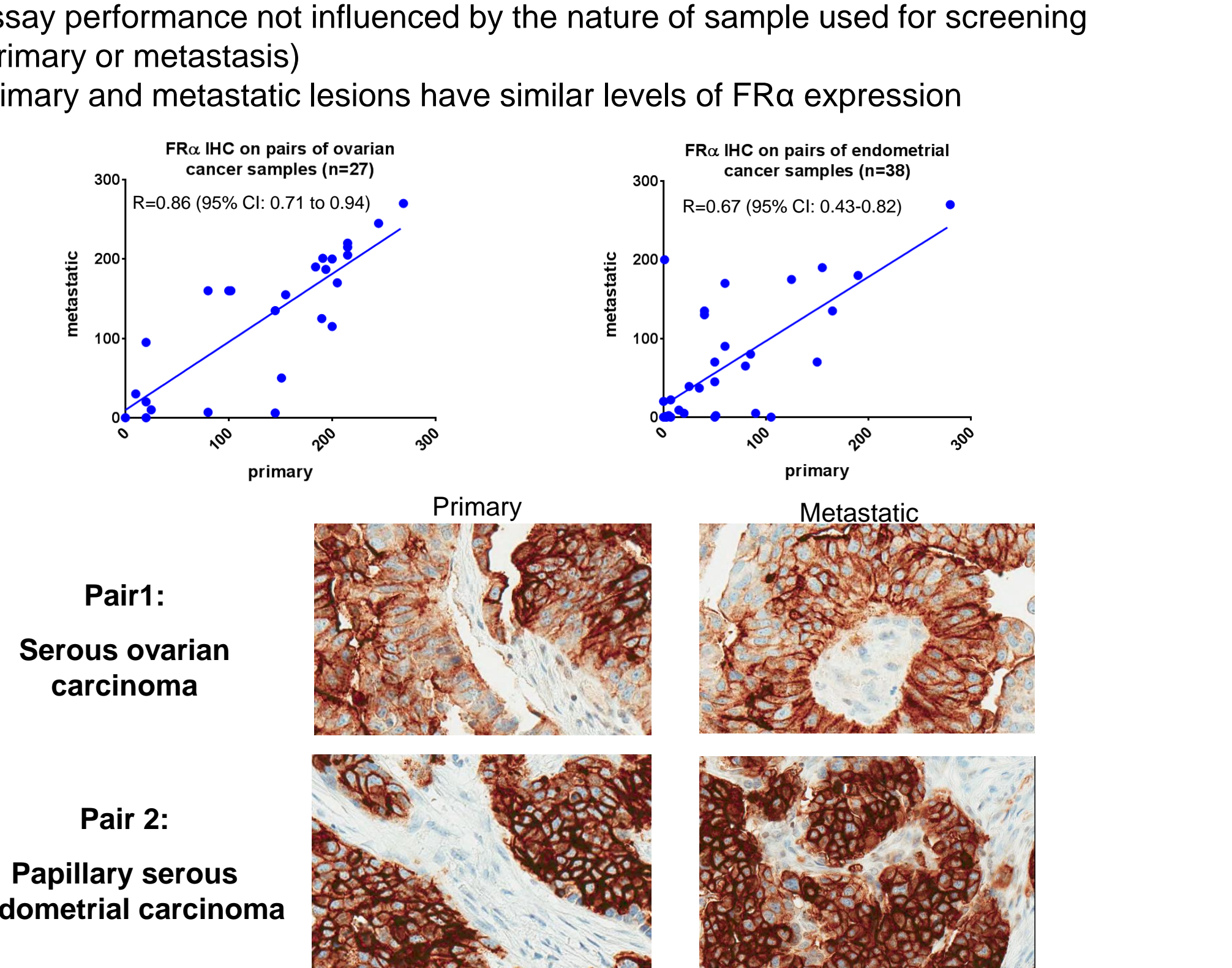- 201-300
- 101-200
- 1-100
- 0

Greater proportion of ovarian cancer (OVC) showed high FRα expression than endometrial cancer (EMC)

Endometrial carcinoma with serous histology more likely to show high FRα expression

FRα expression in histological subtypes of endometrial cancer

serous (n=21)   endometrioid (n=37)   mixed (n=8)   NOS (n=14)

NOS: Not Otherwise Specified

Serous carcinoma (H score=280)   Serous and endometrioid mixed carcinoma (H score=150)   Endometrioid carcinoma (H score=50)

Strong correlation in FRα expression between paired primary and metastatic samples

- Assay performance not influenced by the nature of sample used for screening (primary or metastasis)
- Primary and metastatic lesions have similar levels of FRα expression

FRα IHC on pairs of ovarian cancer samples (n=27)
R=0.86 (95% CI: 0.71 to 0.94)

FRα IHC on pairs of endometrial cancer samples (n=38)
R=0.67 (95% CI: 0.43-0.82)

Primary   Metastatic

Pair 1: Serous ovarian carcinoma

Pair 2: Papillary serous endometrial carcinoma

SUMMARY

1. An immunohistochemistry-compatible FRα-specific antibody was generated and selected from a panel of monoclonal antibodies for its ability to bind to denatured epitopes.
2. A robust IHC assay for detecting FRα expression in archival tumor tissue was developed and analytically validated, supporting patient selection in clinical trials.
3. Staining of tumor samples using this assay demonstrated that a greater proportion of ovarian samples exhibited high FRα expression than endometrial samples.
4. Among endometrial samples, a greater proportion of tumors with the serous histology exhibited high FRα expression than the other histological subtypes.
5. Comparison of paired primary/metastatic lesions from the same patients showed a strong correlation of FRα expression levels between the two, demonstrating consistency and utility of the assay irrespective of the sample source.
6. This assay is currently in use for patient selection in clinical trials for mirvetuximab soravtansine.