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In vitro evaluation of ruxolitinib to target JAK/STAT pathway in a microenvironment that mimics ovarian cancer

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REVIEW ARTICLE | Open Access

Potential drug repurposing of ruxolitinib to inhibit the JAK/STAT pathway for the treatment of patients with epithelial ovarian cancer

Irfan Yunianto, Margaret Currie, Kenny Chitcholtan, Peter Sykes

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Abstract

Aim

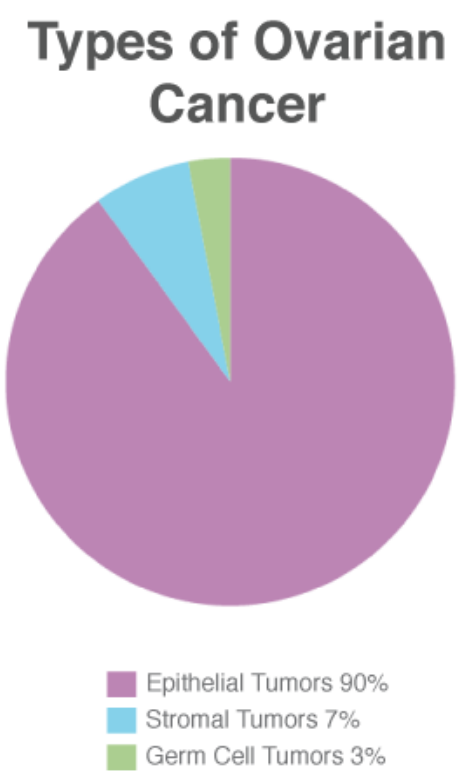
This review aimed to describe the potential for therapeutic targeting of the JAK/STAT signaling pathway by repurposing the clinically-approved JAK inhibitor ruxolitinib in the patients with epithelial ovarian cancer (OC) setting.

Ovarian Cancer (OC)

Ovarian cancer is the **5th** most common cancer in women.

20,000 women will be diagnosed with ovarian cancer each year.

1 in 73 women will develop ovarian cancer in her lifetime.

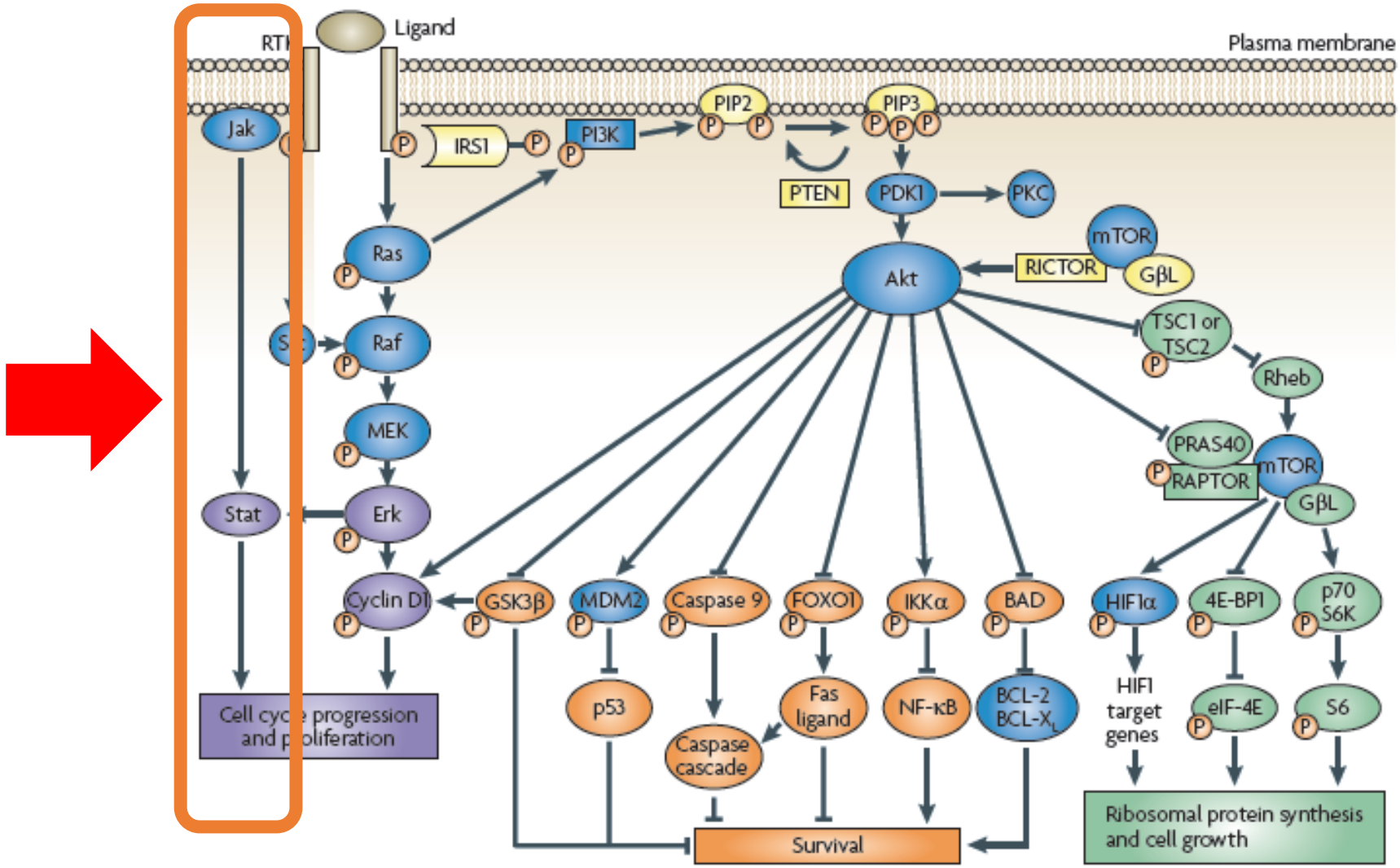


(**U.S. Data**, CDC; Holland, K. 2014. http://www.healthline.com/health/cancer/ovarian-cancer-facts-statistics-infographic#1)

- OC was the 9th most commonly registered cancer for females, and 5th leading cause of cancer death in NZ women in 2021
- Recurrent OC remains a major challenge since there is >80% patient mortality within 5 years

(www.health.govt.nz/publication/new-cancer-registrations-2019; Ahmed et al. Cells 2020, 9, 719; doi:10.3390/cells9030719)

Key signaling pathways involved in the progression of ovarian cancer and potential targets for anticancer therapy



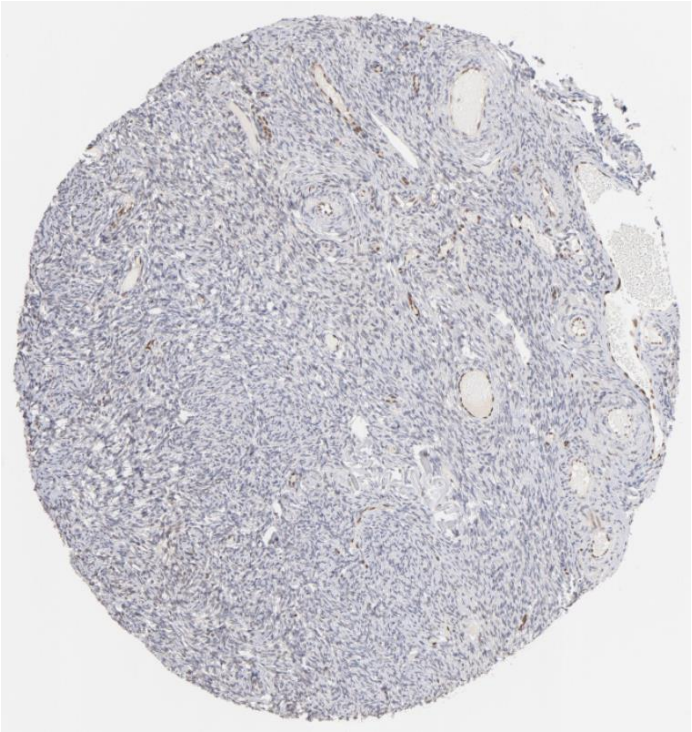
Proteins shown in blue represent current and new drug targets for ovarian cancer

Rybak et al. 2014. DOI: 10.18632/oncotarget.2953

Role of JAK2/STAT3 Pathway in Ovarian Carcinogenesis

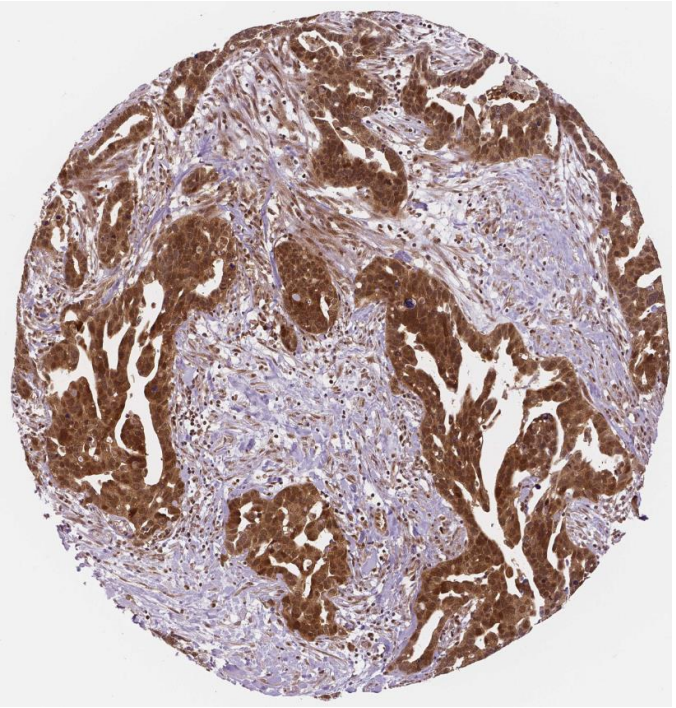
- Constitutive Activation of STAT3 is frequently detected in OC → tumour proliferation, survival, invasion and angiogenesis, suppress antitumour immune response, support tumour –promoting inflammation
- STAT3 is activated by Janus family kinases (JAK) via cytokine receptors, growth factor receptors, and non-growth factor receptor tyrosine kinases
- Direct therapeutic targeting of STAT3 is **challenging** → lack of drug-targetable intrinsic catalytic activity of the protein, bioavailability
- Targeting JAK → clinically relevant, and plausible approach
- Repurposing **Ruxolitinib** (ruxo, JAK1/2 inhibitor) → potential therapeutic effects in OC
- Ascites fluid → hallmark of late-stage OC → Tumour Microenvironment (TME)

Mol Cancer Ther; 14(4) April 2015



Ovarian Stroma cells
Female, age 60
Ovary, normal tissue
STAT3 staining: low
Intensity: weak
Quantity: 75%-25%
Loc: nuclear

(www.proteinatlas.org)



Ovarian Cancer
Female, age 54
Ovary, cystadenocarcinoma, serous
STAT3 staining: high
Intensity: strong
Quantity: >75%
Loc: cytoplasmic/membranous, nuclear

Why ruxolitinib?

- FDA-approved for MF → potential to repurpose in OC setting
- effectively inhibit the pSTAT3 in OVCAR8, SKOV3, and MDAH2774 cells, reduced cell viability with IC_{50} value in the range of 10 to 17 mM.
- synergistically increased antitumor activity of cisplatin, carboplatin, paclitaxel, doxorubicin, and topotecan. The IC_{50} of these anticancer agents decreased two- to threefolds in the presence of ruxolitinib.
- reduced the tumor burden of OVCAR8 in a peritoneal ovarian cancer mouse model

(Han et al, 2016, 2018; Reeves et al., 2017)

Previous works on JAKi in OC

Cancer Biology and Signal Transduction

Molecular Cancer Therapeutics

Targeted Blockade of JAK/STAT3 Signaling Inhibits Ovarian Carcinoma Growth

doi: 10.1158/1535-7163.MCT-14-0800

Galina Gritsina[1], Fang Xiao[1], Shane W. O'Brien[1], Rashid Gabbasov[1,2], Marisa A. Maglaty[1], Ren-Huan Xu[3], Roshan J. Thapa[3], Yan Zhou[4], Emmanuelle Nicolas[5], Samuel Litwin[4], Siddharth Balachandran[3], Luis J. Sigal[3], Dennis Huszar[6], and Denise C. Connolly[1]

Research gaps:

1. **Conducted in 3D culture models?**
2. **Ascitic fluid?**

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2018

Ruxolitinib synergistically enhances the anti-tumor activity of paclitaxel in human ovarian cancer

Ernest S. Han[1], Wei Wen[1,2], Thanh H. Dellinger[1], Jun Wu[3], Selena A. Lu[1], Richard Jove[2,4] and John H. Yim[1]

Translational Oncology
www.transonc.com

Volume 12 Number 8 August 2019 pp. 1015–1025 1015

Increasing Antitumor Activity of JAK Inhibitor by Simultaneous Blocking Multiple Survival Signaling Pathways in Human Ovarian Cancer

Wei Wen*†, Ernest S. Han†, Thanh H. Dellinger†, Jun Wu‡, Yuming Guo‡, Ralf Buettner*, David A. Horne*, Richard Jove*† and John H. Yim†

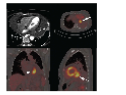
*Department of Molecular Medicine, Beckman Research Institute, City of Hope Comprehensive Cancer Center, 1500 East Duarte Rd., Duarte, CA 91010; †Department of Surgery, Beckman Research Institute, City of Hope Comprehensive Cancer Center, 1500 East Duarte Rd., Duarte, CA 91010; ‡Department of Comparative Medicine, Beckman Research Institute, City of Hope Comprehensive Cancer Center, 1500 East Duarte Rd., Duarte, CA 91010

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 55), pp: 94040-94053

Research Paper

Ruxolitinib sensitizes ovarian cancer to reduced dose Taxol, limits tumor growth and improves survival in immune competent mice

Patrick M. Reeves[1], Mojgan A. Abbaslou[1], Farah R.W. Kools[1], Kritchai Vutipongsatorn[1], Xiaoyun Tong[1], Christina Gavegano[2], Raymond F. Schinazi[2] and Mark C. Poznansky[1]



Cancer Biology & Therapy

ISSN: 1538-4047 (Print) 1555-8576 (Online) Journal homepage: http://www.tandfonline.com/loi/kcbt20

Relationship between HER2 and JAK/STAT-SOCS3 signaling pathway and clinicopathological features and prognosis of ovarian cancer

An-Quan Shang, Jian Wu, Feng Bi, Yu-Jie Zhang, Lei-Rong Xu, Ling-Ling Li, Fei-Fei Chen, Wei-Wei Wang, Jian-Jun Zhu & You-Yi Liu

Hypotheses :

1. Inhibition of JAK2/STAT3 by ruxolitinib effectively reduce OC cell viability
2. Malignant ascitic fluid attenuates the effectiveness of ruxolitinib treatment in 2D and 3D cell culture models
3. The 3D culture model of OC cells compromise ruxolitinib treatment in reducing OC cell viability

Methods

Human OC cell lines (SKOV3 and OV90), Mouse cell line ID8

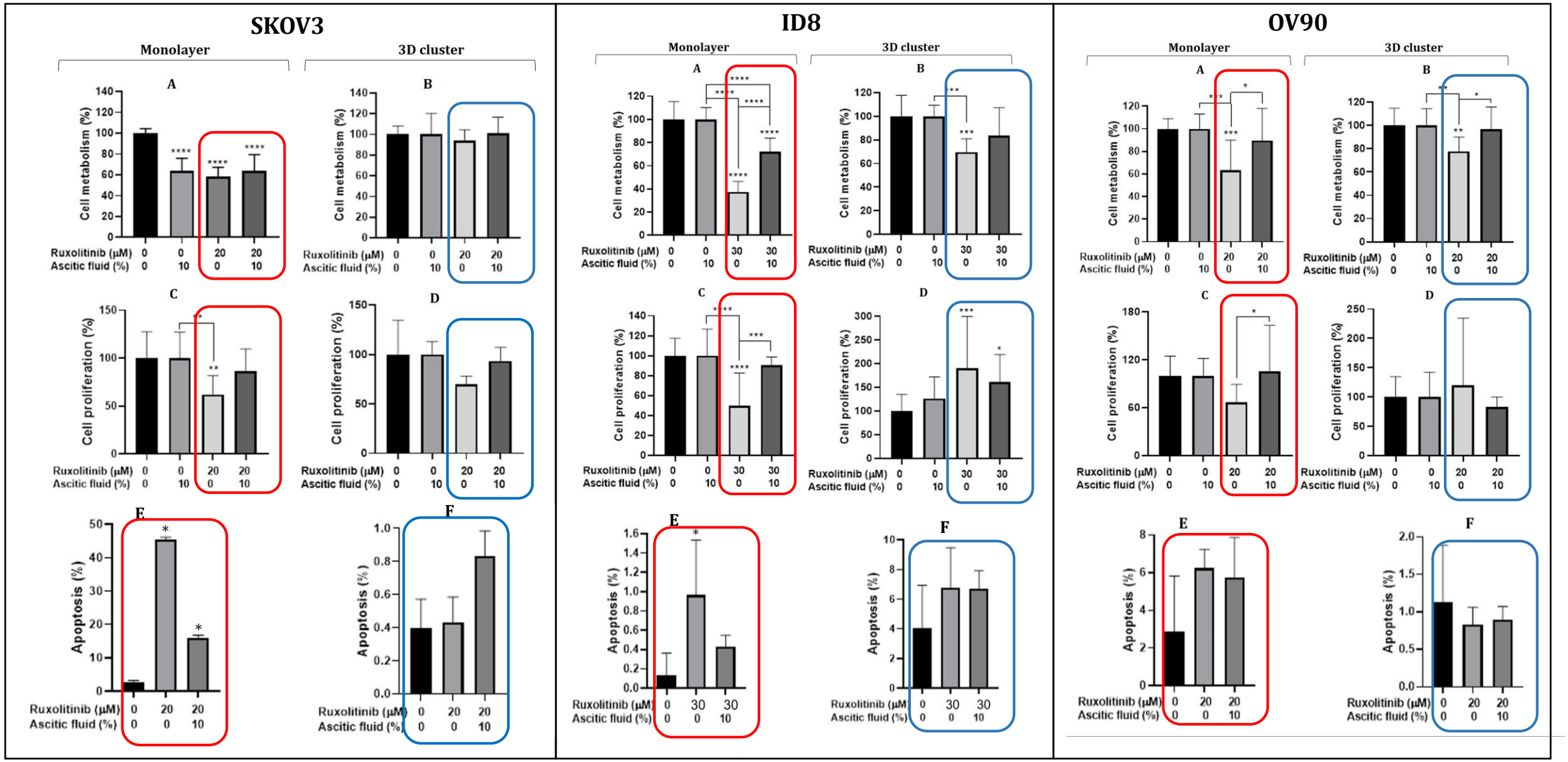
Monolayer model

3D cluster model

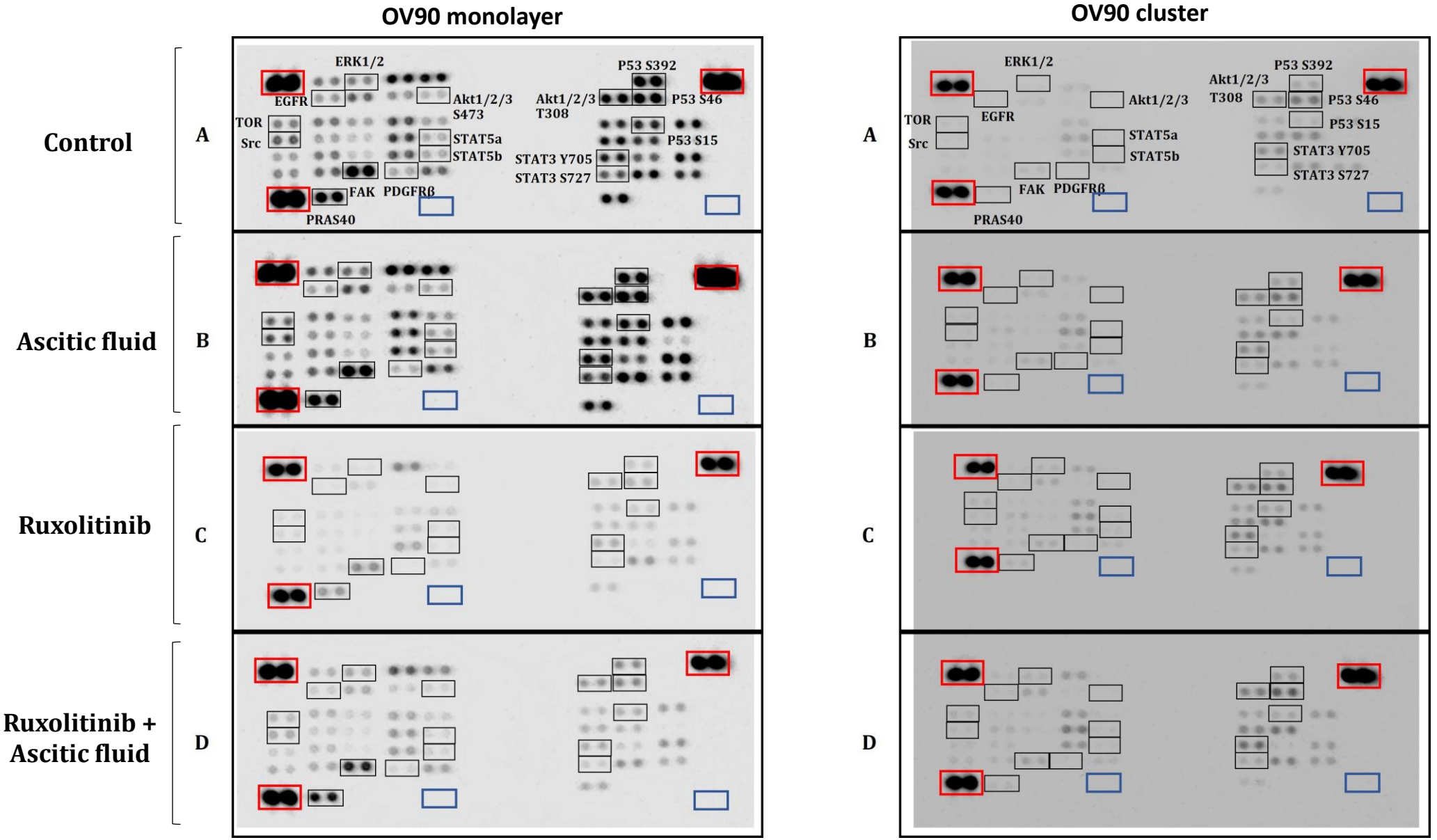
ruxolitinib 20 µM (SKOV3 and OV90); ruxolitinib 30 µM (ID8);
no ascitic fluid; 10% ascitic fluid; treated for 72 hours

- Cell metabolism, proliferation, apoptosis → Alamar blue, CyQuant NF, Annexin V
- Phosphorylation levels → JAK/STAT assoc proteins in OV90 → Human phospho-kinase array proteomic profiler (R&D Systems, ARY003B)
- Pathway enrichment analysis → IMPaLA (Integrated Molecular Pathway Level Analysis) → biomolecular justification → potential pathway to target in combo with ruxo

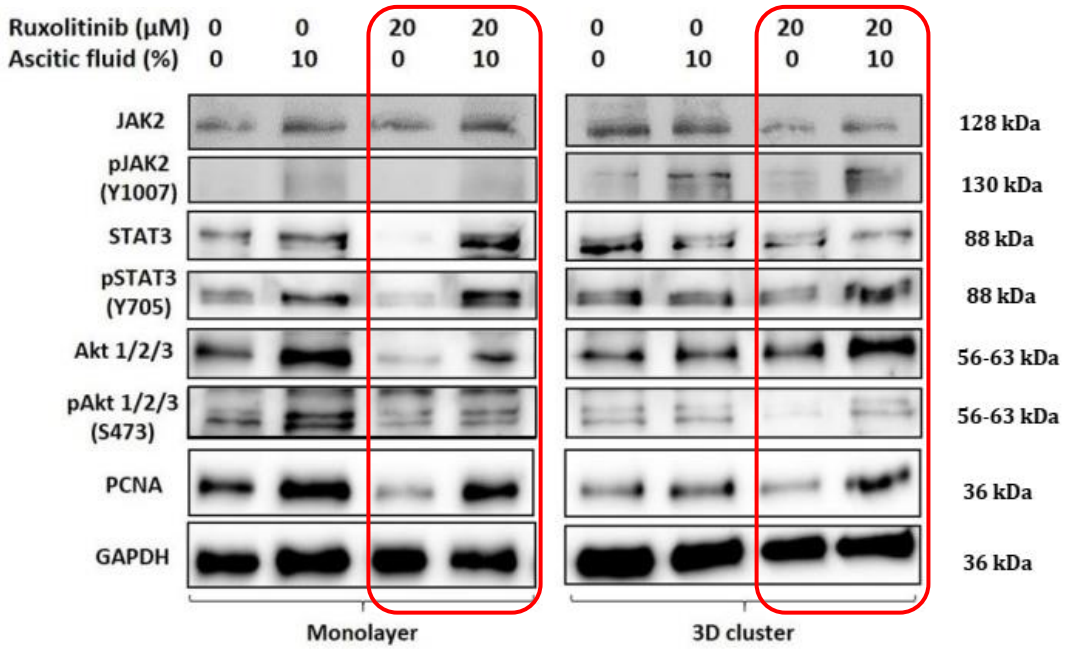
The effects of ruxo and ascitic fluid on OC cells viability and apoptosis

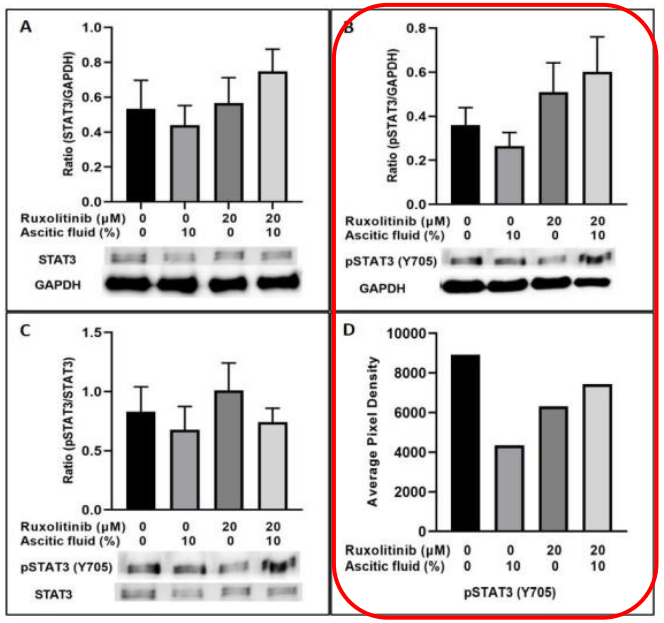


Human phospho-kinase proteomic array profile



Validation of OV90 proteins of interest using Western analysis





3D cluster

Conclusion

- Ruxolitinib has limited activity in 3D culture, and ascitic fluid further attenuated its efficacy.
- Phospho-kinase proteomic array and the pathway enrichment analysis showed alternative activated compensatory pathway due to ruxo and ascitic fluid treatment → biomolecular justification for the potential use of combination targeted therapy
- The importance of 3D cell culture and the TME in drug sensitivity assays

THANK YOU!

Acknowledgement

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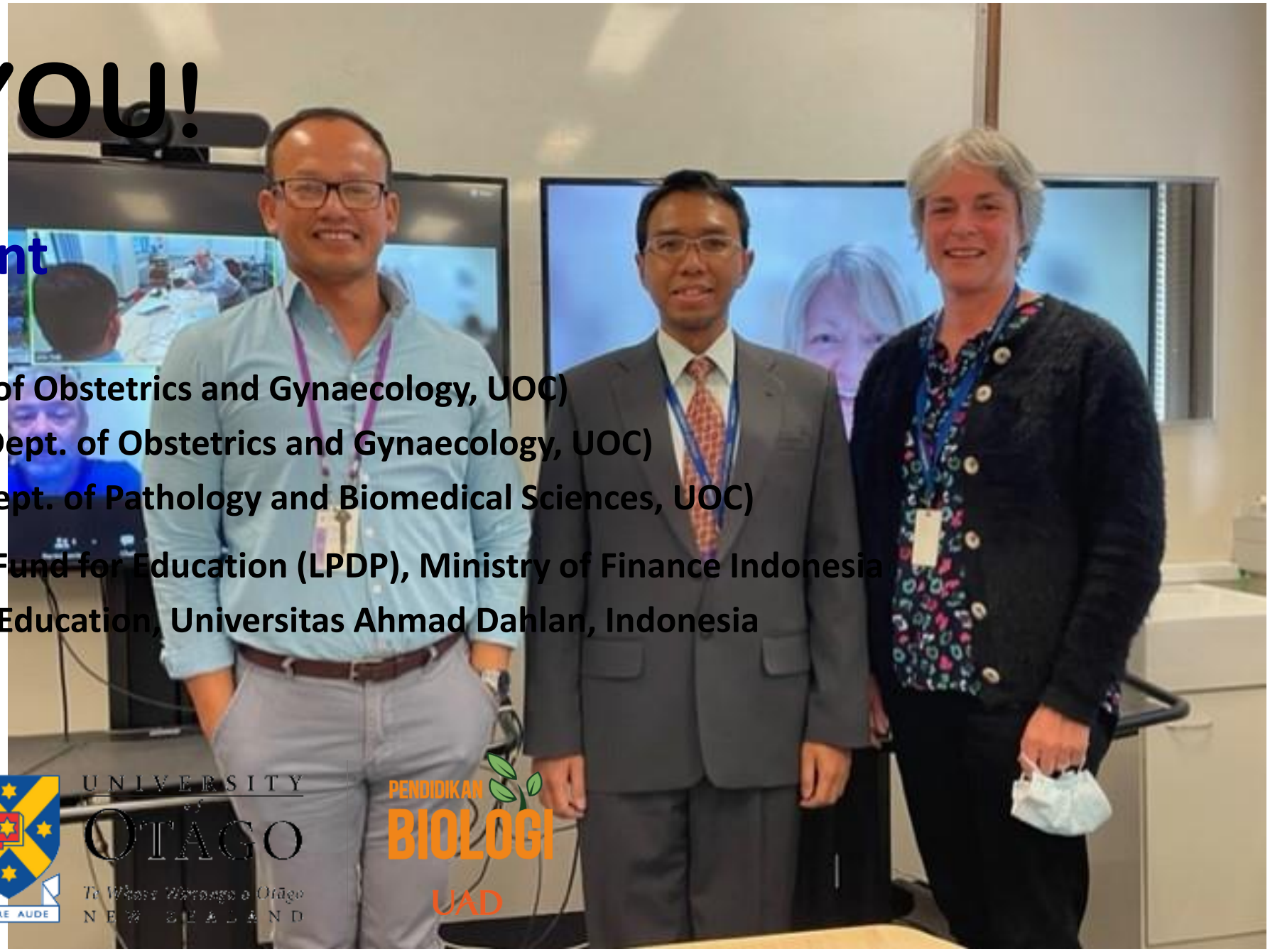
- Department of Biology Education, Universitas Ahmad Dahlan, Indonesia



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