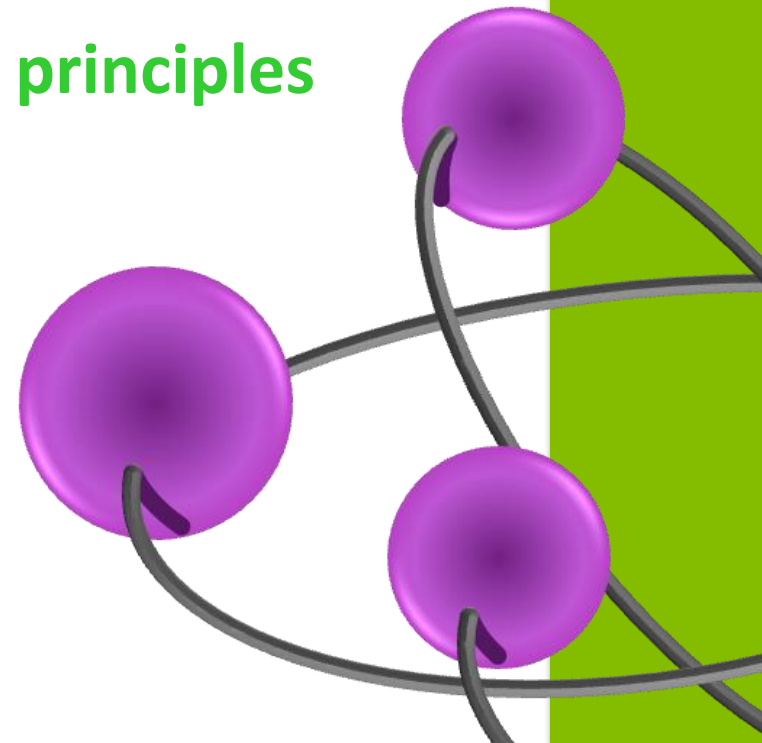





ROR1 – a novel RTK for onco- targeting monoclonal antibodies of small molecules

Towards new therapeutic principles

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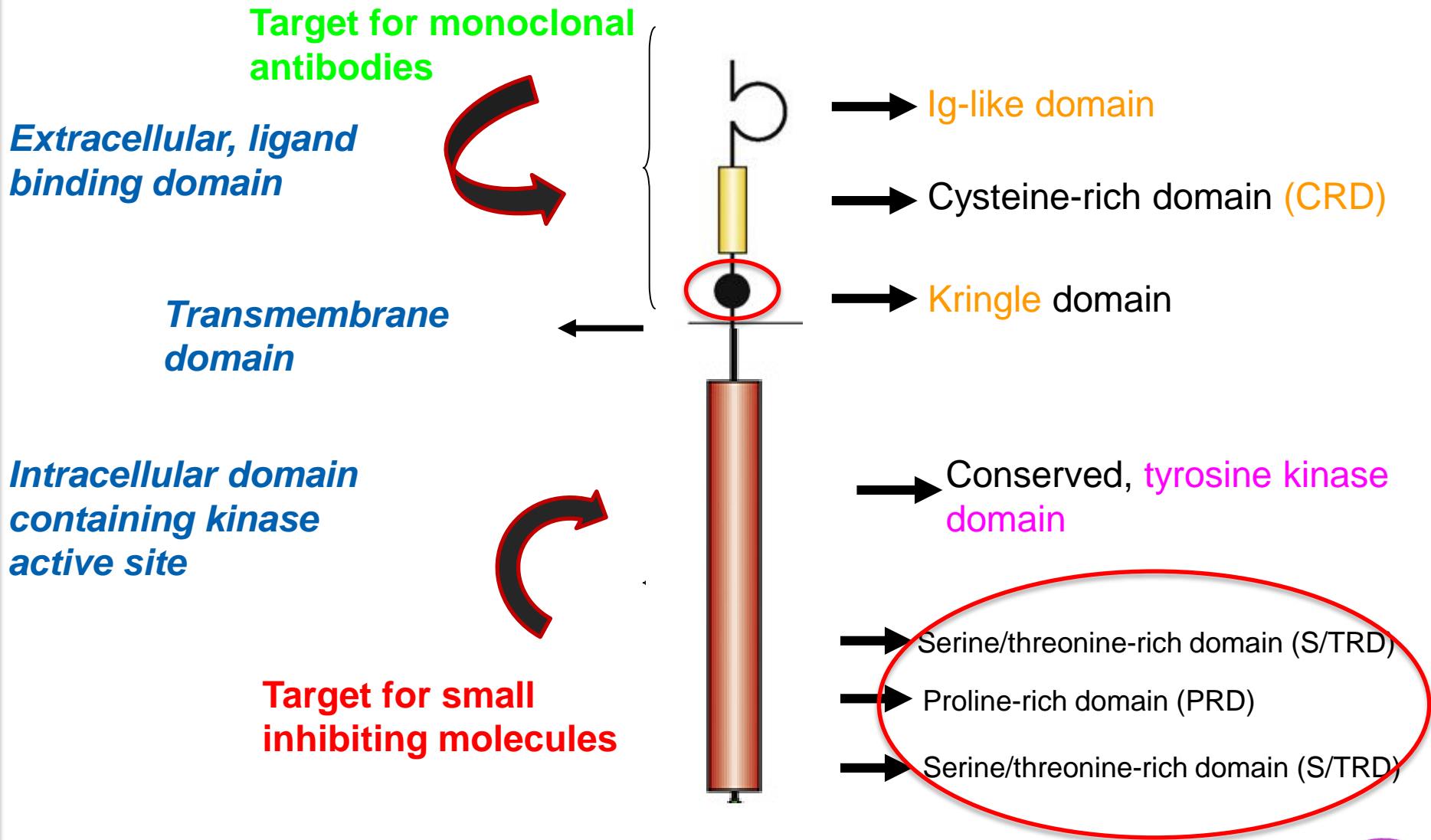




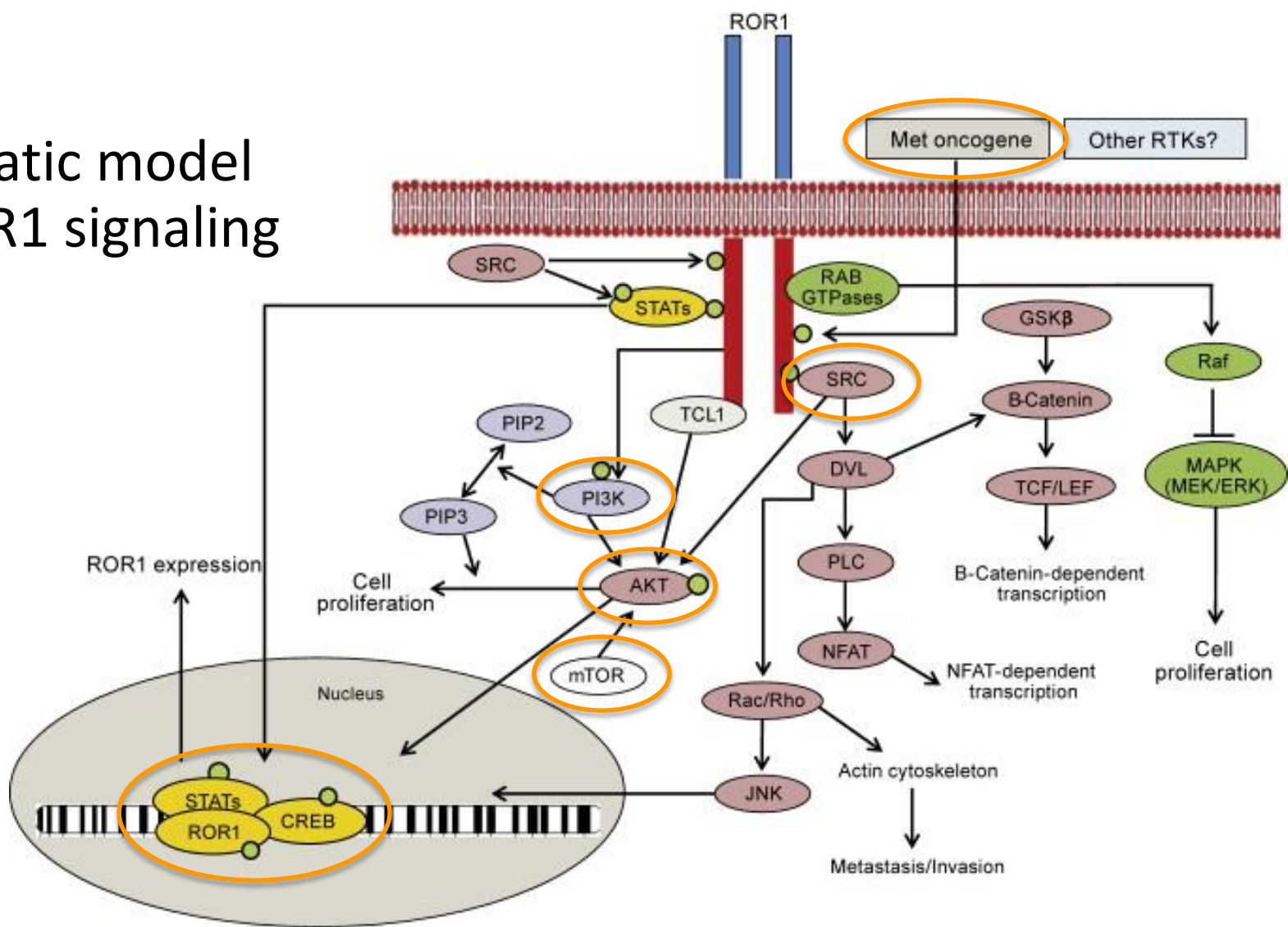
Receptor tyrosine kinase-like orphan receptor (ROR1)

- ROR1 - 2814 nucleotides on chromosome 1 (1p31-32)
- 937 amino acids, with a size of 105-130 kDa; various isoforms have been described e.g. 64 kDa
- Expressed in embryonic tissues but absent in adult tissues of importance for cell differentiation, migration, survival and organogenesis
- Deletion of ROR1 in mice might be lethal, associated with pulmonary, skeletal and cardiac defects

ROR1 Structure

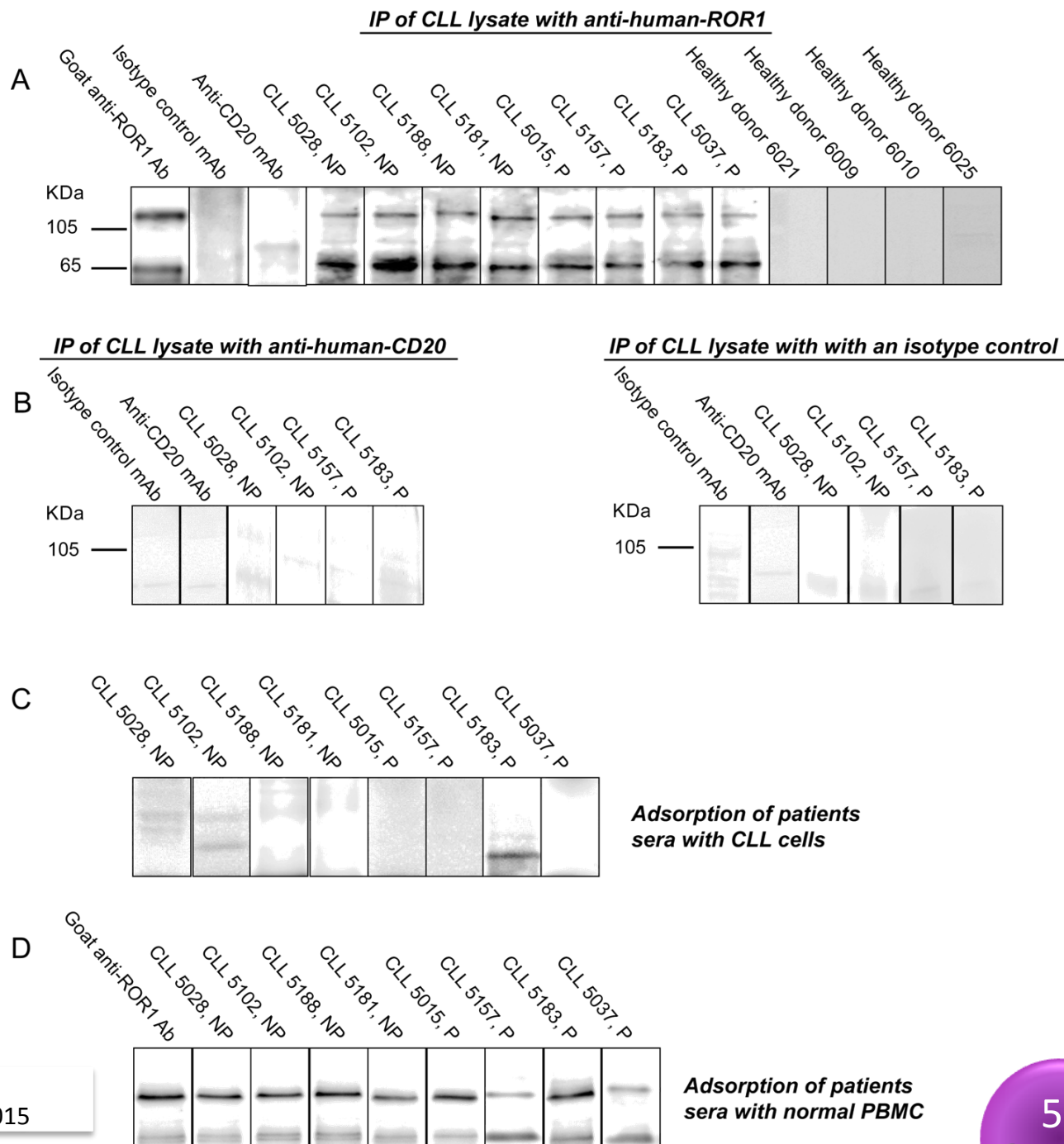


Schematic model for ROR1 signaling

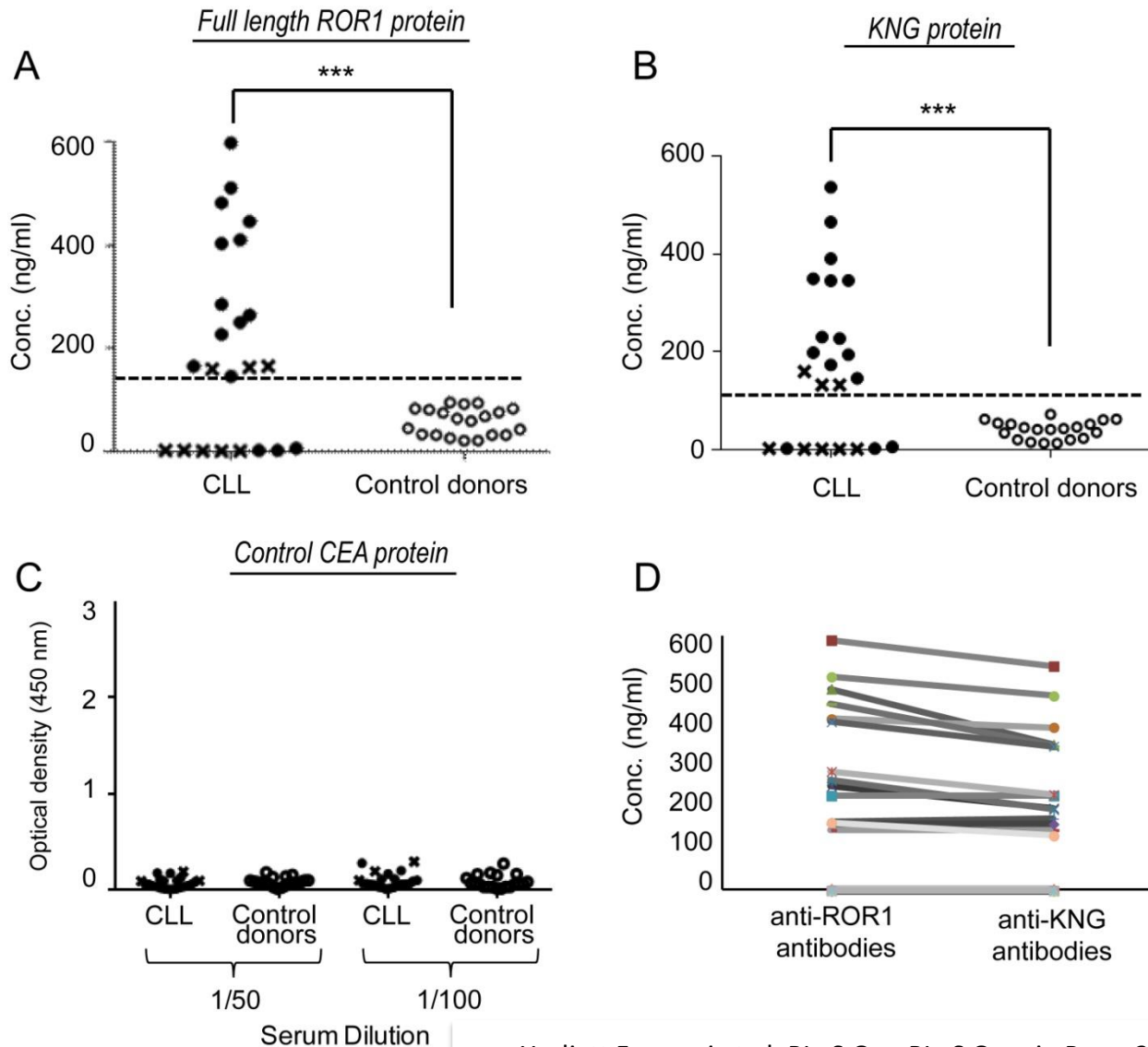


The ROR1 receptor tyrosine kinase recruits canonical and non-canonical signaling **pathways for cell survival and invasion**. A central pathway is the PI3K/AKT/mTOR pathway which activates the CREB transcription factor for nucleus translocation. ROR1 kinase-dependent SRC activation is a key initiating event and ROR1 acts also as a MET substrate. Proteins like ROR1, STATs and CREB might act as transcription factors and bind to ROR1 promoter region to enhance the expression of the *ROR1* gene. Phosphate groups are denoted as green circles.

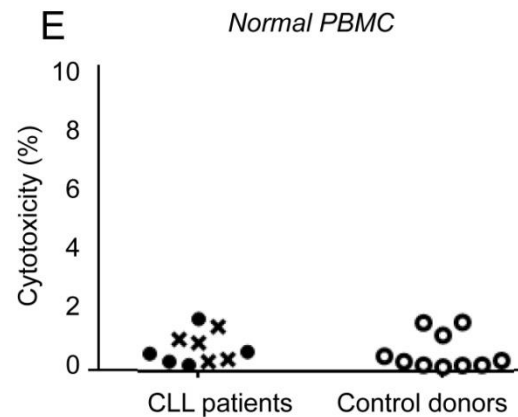
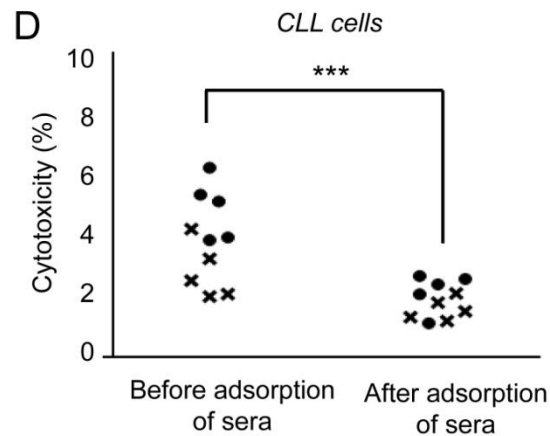
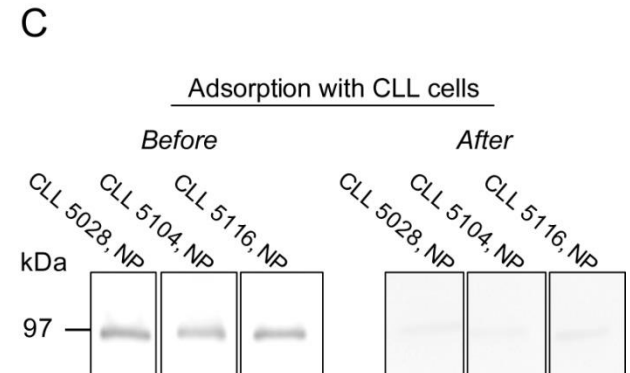
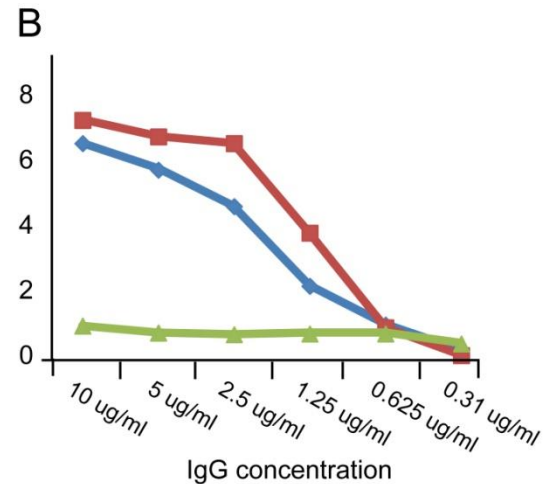
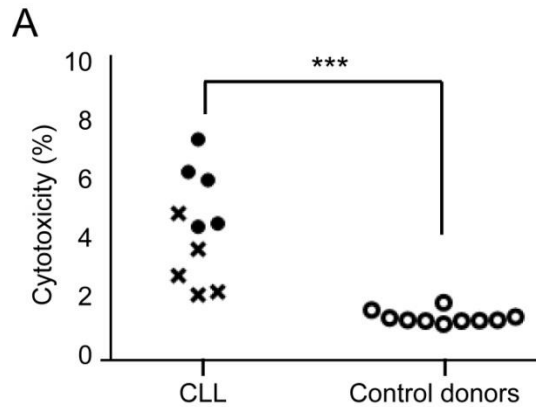
Patients with CLL develop spontaneously anti-ROR1 antibodies



Spontaneously induced antibodies in CLL patients against the full-length ROR1 protein and the KNG domain in ELISA



Spontaneously induced anti-ROR1 antibodies in CLL patients were cytotoxic for CLL cells

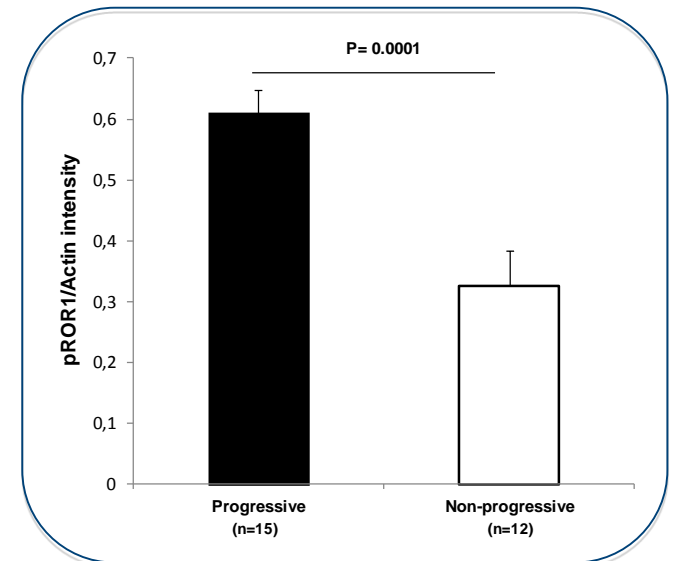


ROR1 as biomarker and therapeutic target

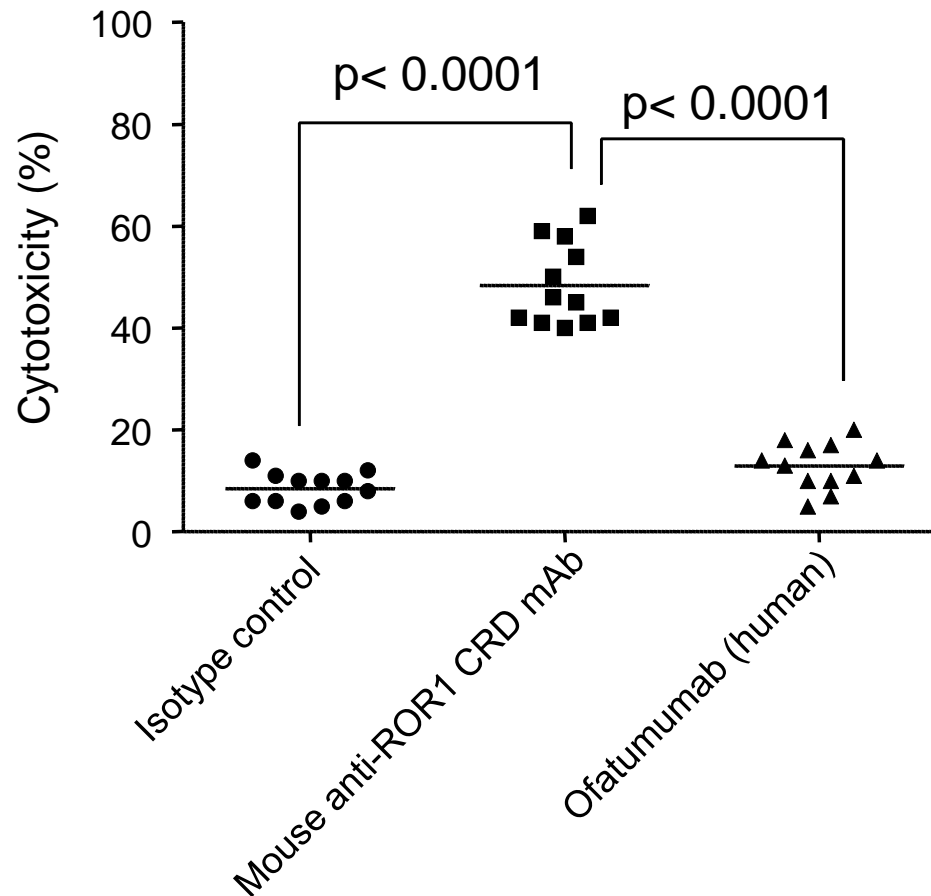
ROR1 expression intensity associated with aggressive disease in CLL as well as in pancreatic, breast, lung and ovarian carcinoma

- Leukemia: Mellstedt et al, Leuk Lymphoma. Apr;54(4):843-50 2013
- Pancreatic cancer: Manuela Iezzi, University Chieti-Pescara, personal communication to Prof. Mellstedt
- Breast cancer: Zhang et al, PLoS One 7,(3) e31127, 2012
- Ovarian cancer: Zhang et al, Am J Pathol. 181, 1903, 2012; Zhang et al, PNAS, Nov 19, 2014 (Epub ahead)
- Lung cancer: Karachaliou et al, Translat Lung Cancer Res, 3(3):122-130, 2014

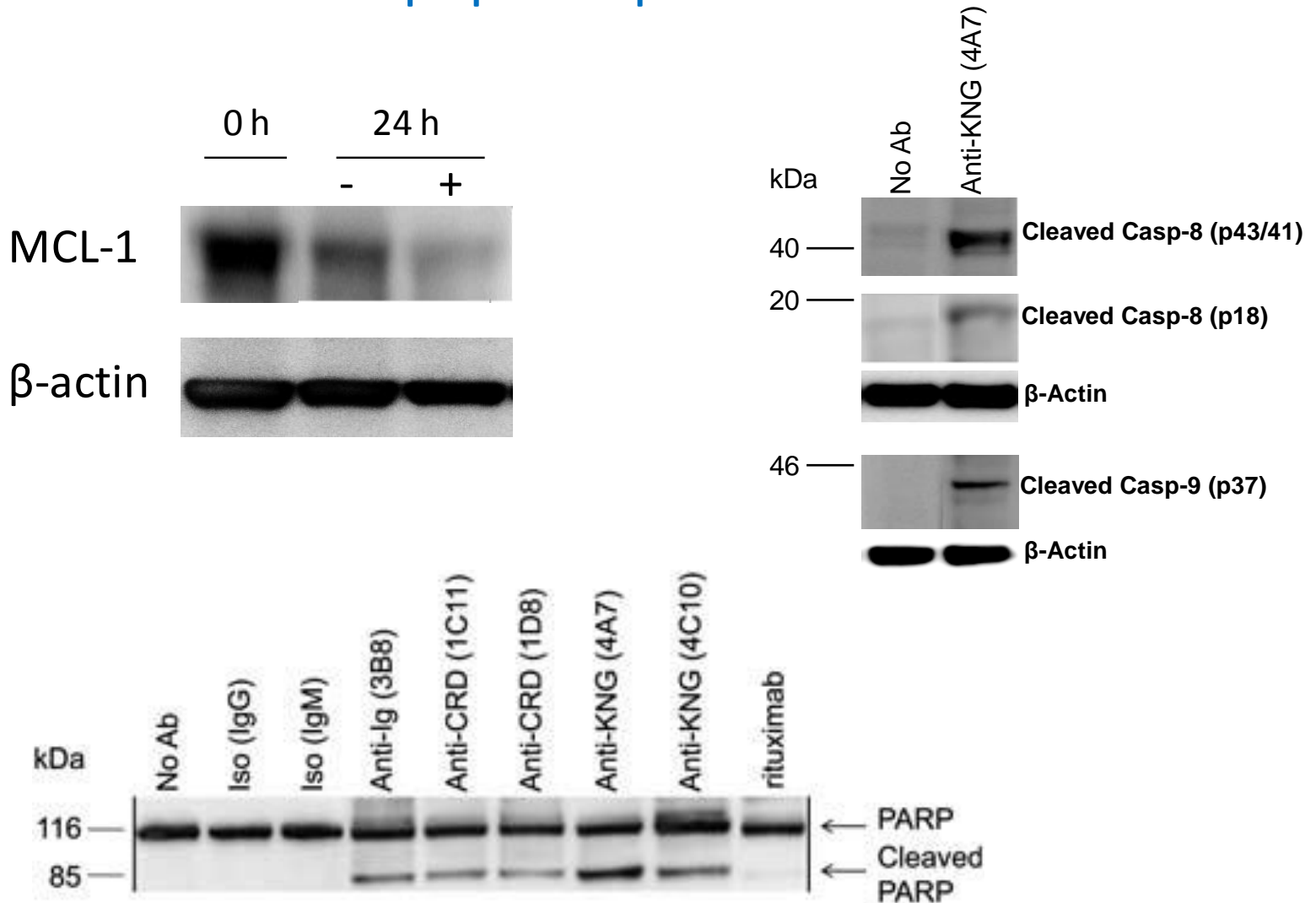
- ROR1 phosphorylation intensity associated with disease activity in CLL
- ROR1 siRNA transfection induced specific cell death of CLL, pancreatic, breast, lung, and ovarian cancer cells



Cytotoxicity of mouse anti-CRD ROR1 alone in CLL cells compared to ofatumumab

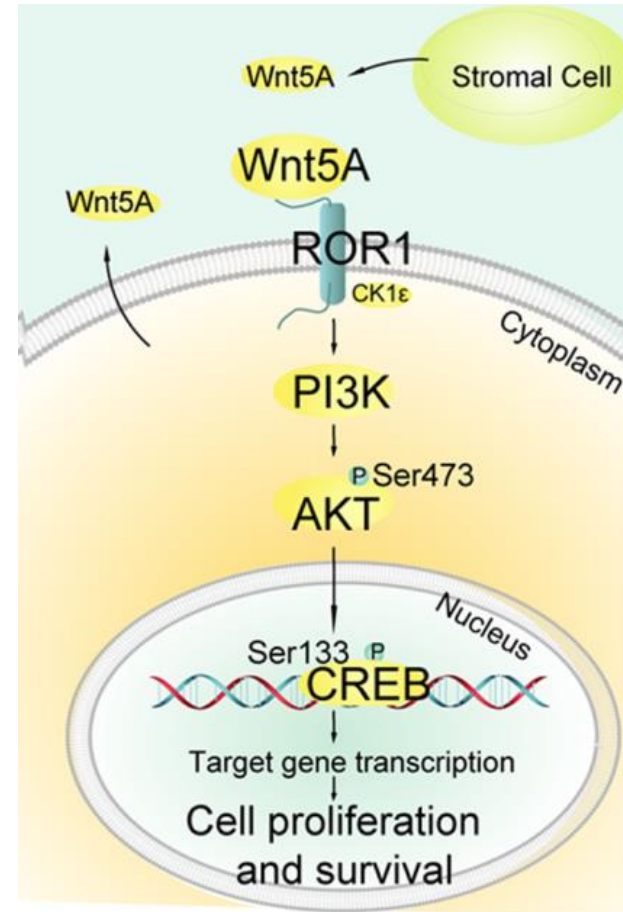
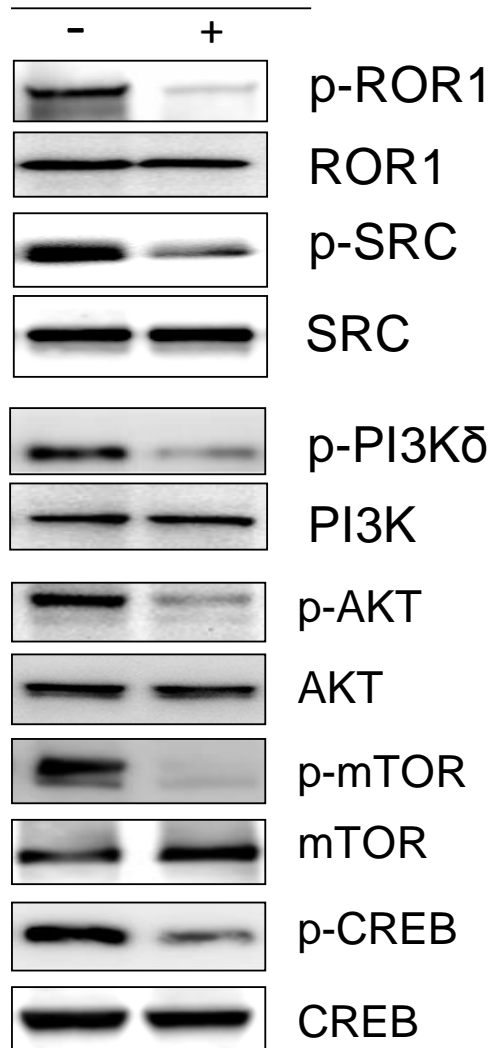


Effect of mouse anti-ROR1 MAbs on apoptotic and anti-apoptotic proteins in CLL cells

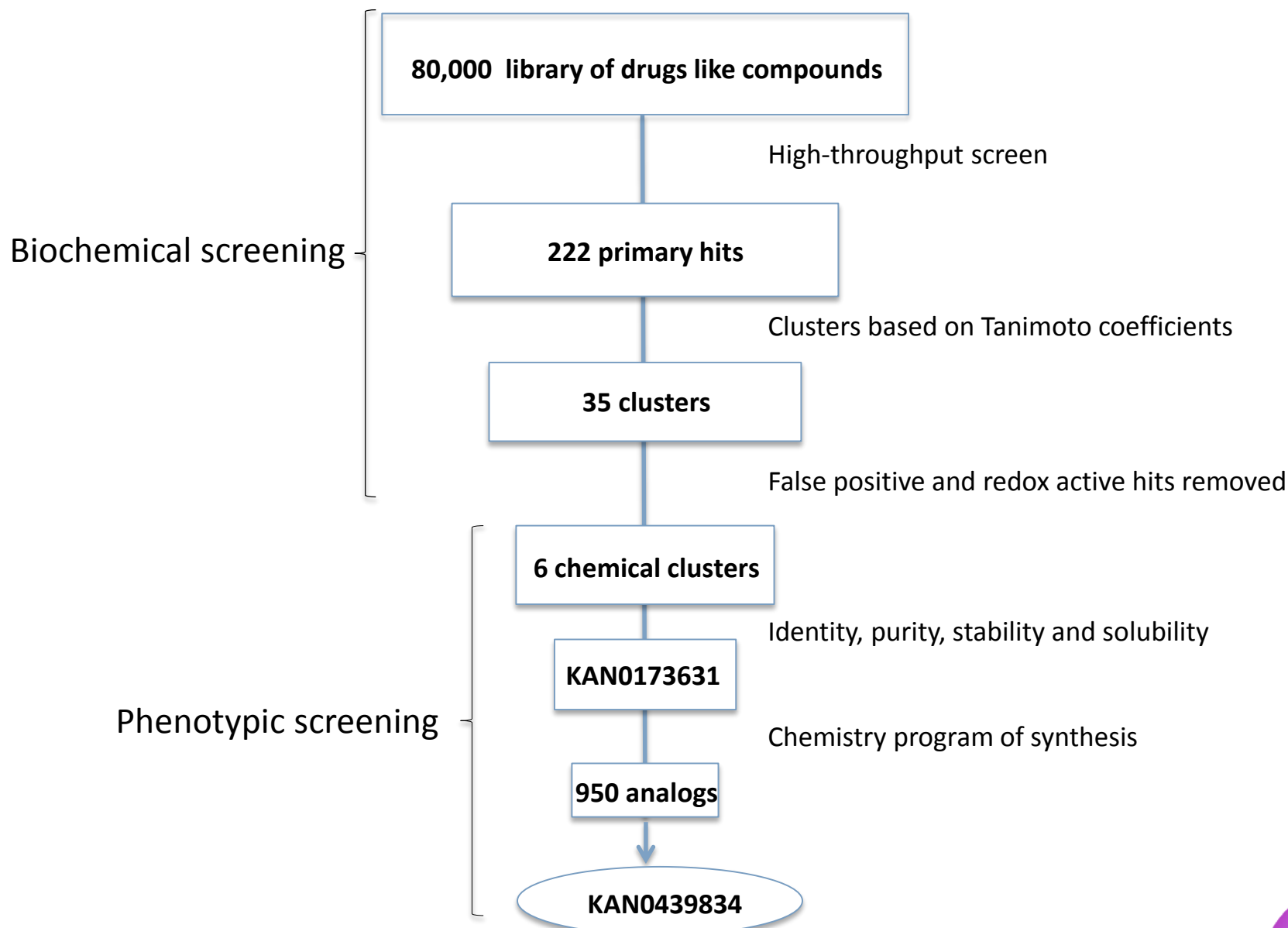


Mouse anti-ROR1 CRD MAb induced dephosphorylation of ROR1 and of downstream signaling molecules in CLL cells

Anti-ROR1 CRD mAb

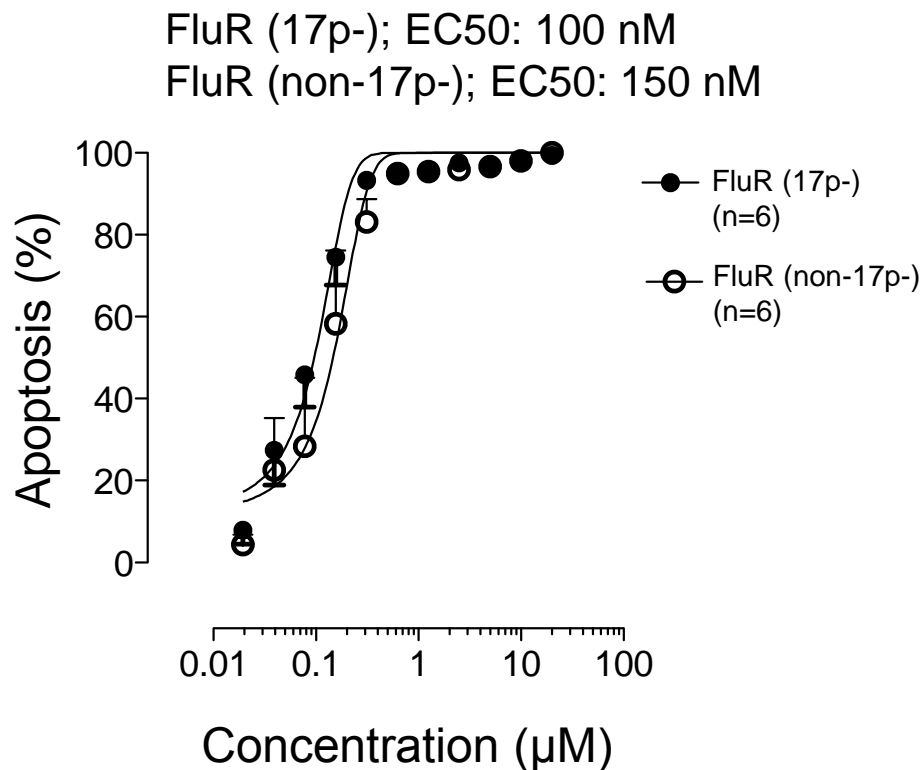
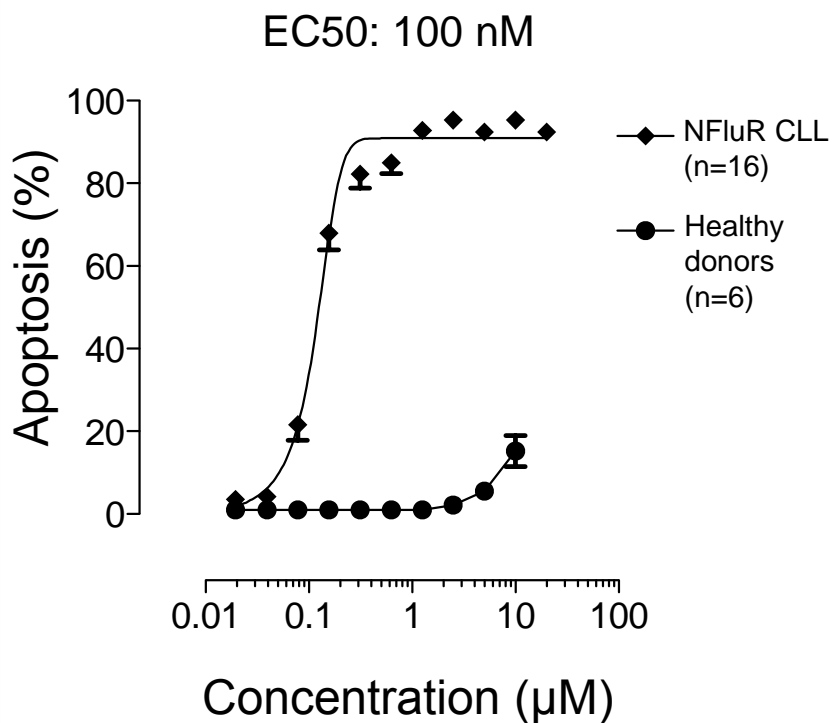


Schematic presentation of the strategy for selection and synthesis of ROR1 tyrosine kinase inhibitors

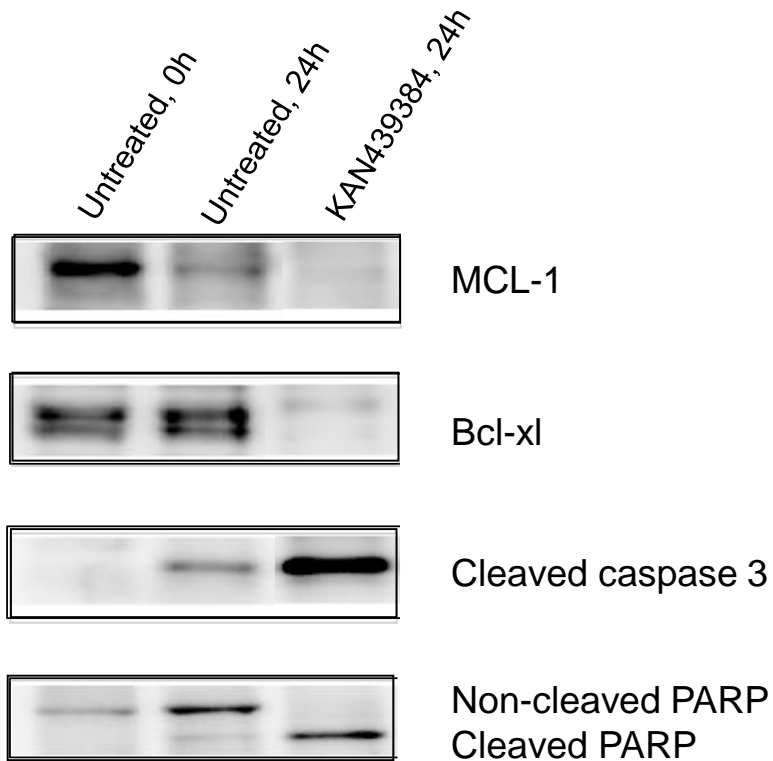


No apparent toxicophores, 530 g/mol molecular mass, metabolically stable in human hepatocytes

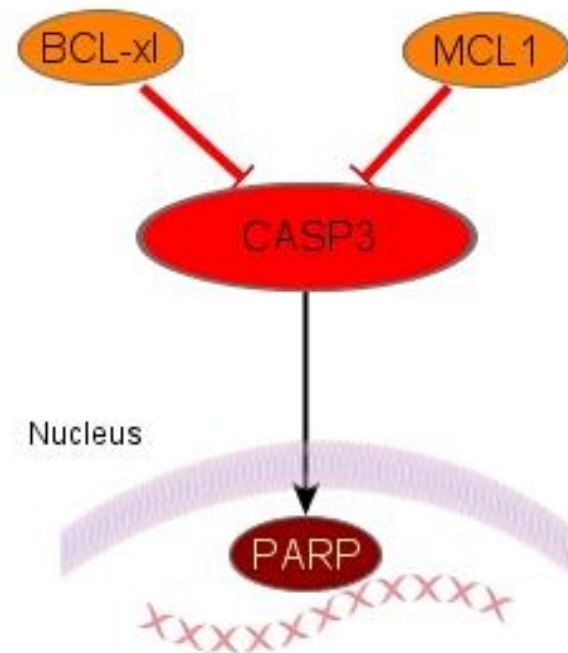
Apoptosis induced by ROR-1 TKI KAN0439834 in non-fludarabine resistant (NFluR) and fludarabine resistant (FluR) CLL cells from patients with and without 17p deletion as well as in healthy donor PBMC



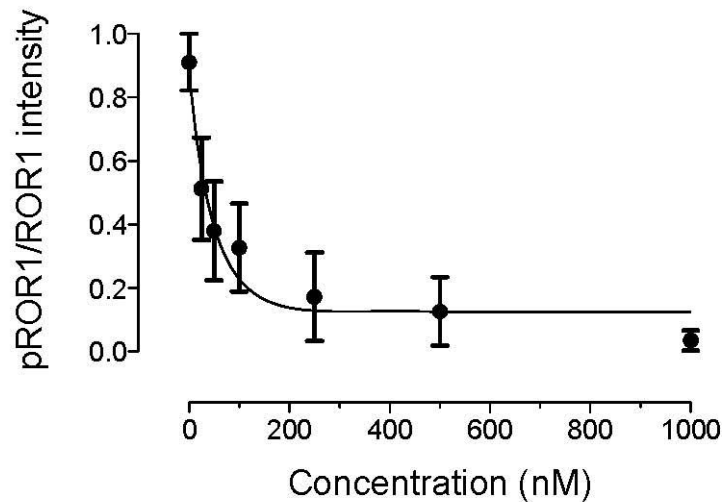
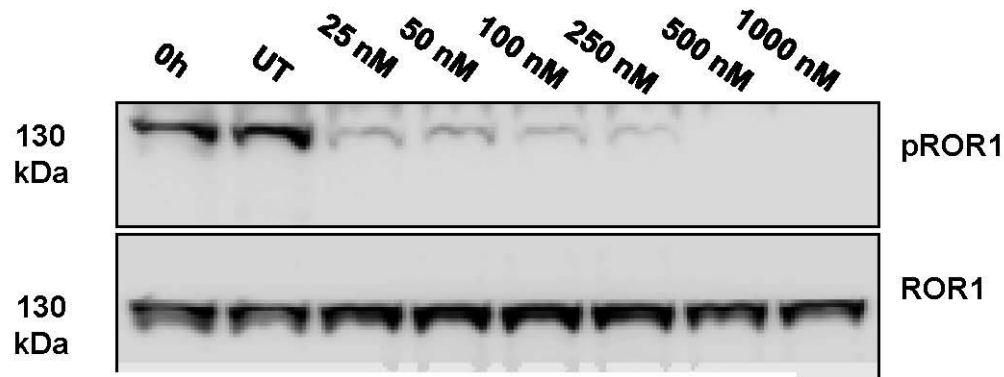
Effect of ROR1 TKI KAN0439834 on apoptotic and anti-apoptotic proteins in CLL cells



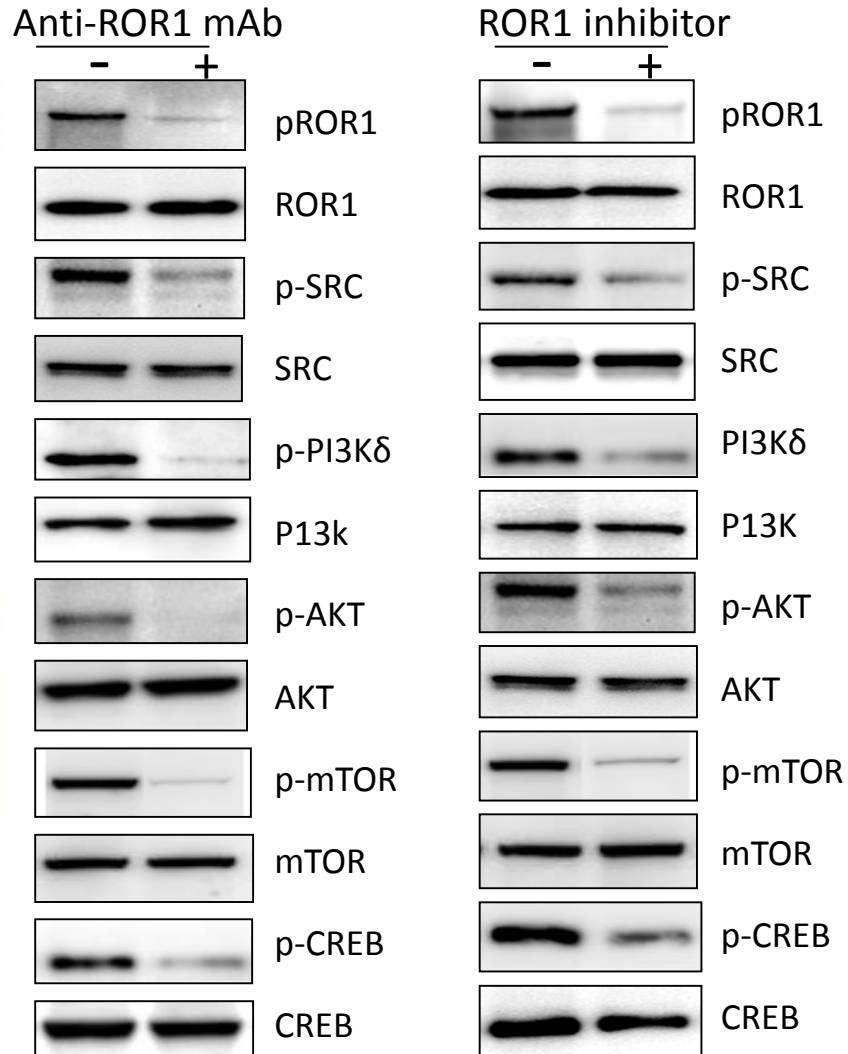
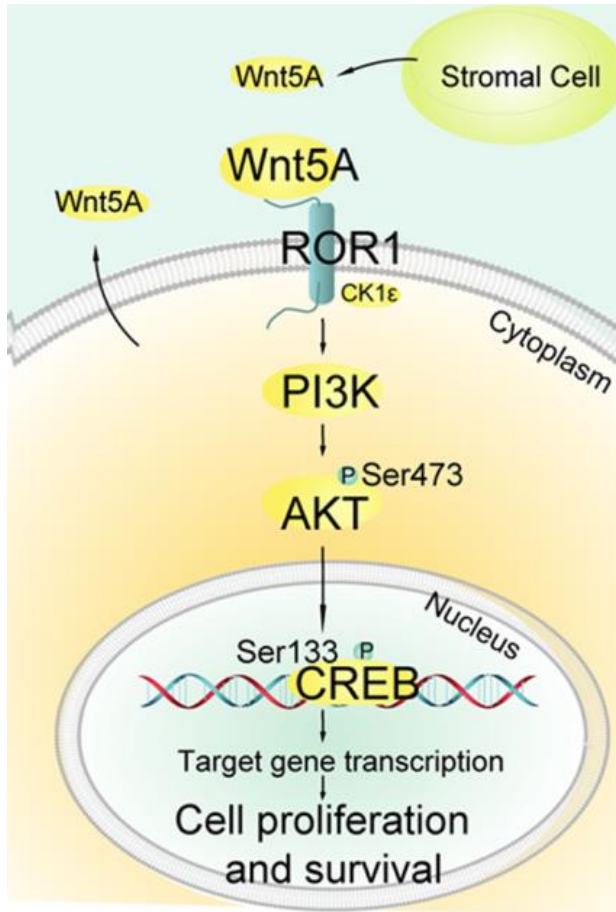
Cytoplasm



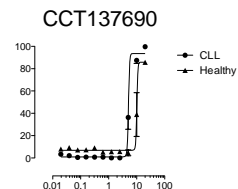
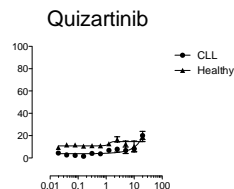
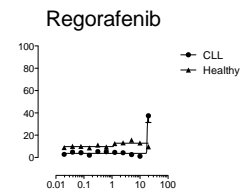
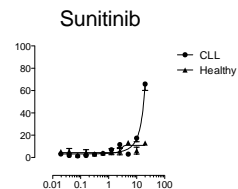
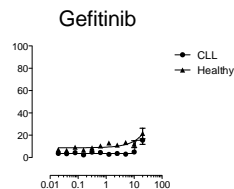
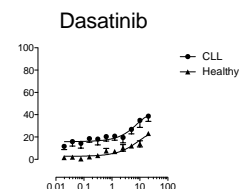
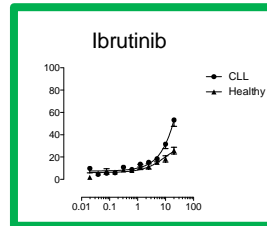
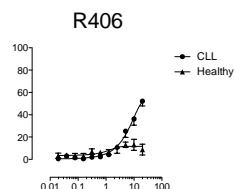
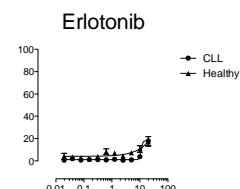
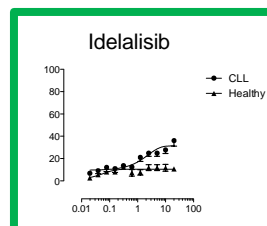
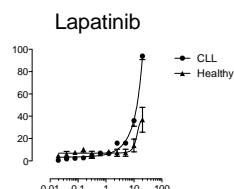
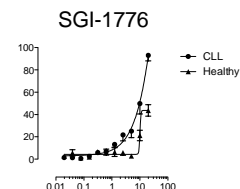
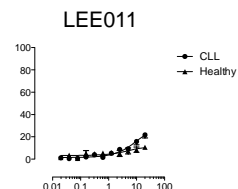
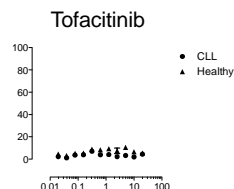
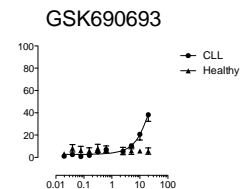
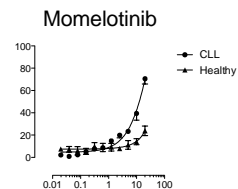
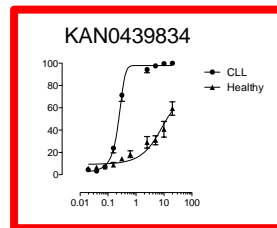
ROR1 TKI KAN0439834 showed fast engagement of the target – 15 min at 25 nM



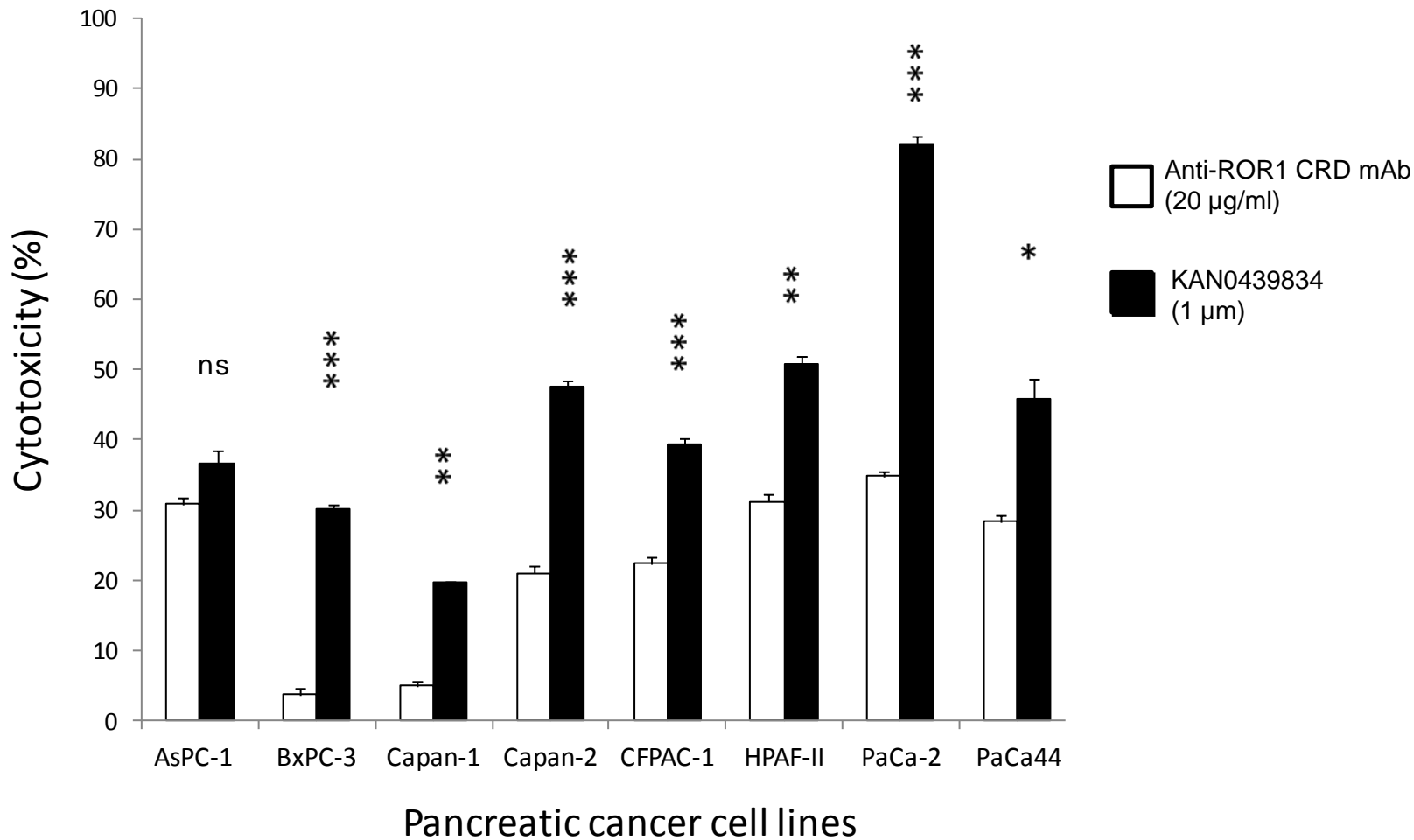
Mouse anti-ROR1 mAb and small molecule ROR1 inhibitor intervened with the non-canonical Wnt signaling pathway in CLL cells



Cytotoxicity (mean \pm SD) of ROR1 inhibitor compared to other kinase inhibitors using CLL cells (n=8) and normal PBMC (n=4).



Cytotoxicity (MTT) of mouse anti-ROR1 CRD mAb and ROR1 TKI KAN0439834 (72 h) in pancreatic cancer cell lines

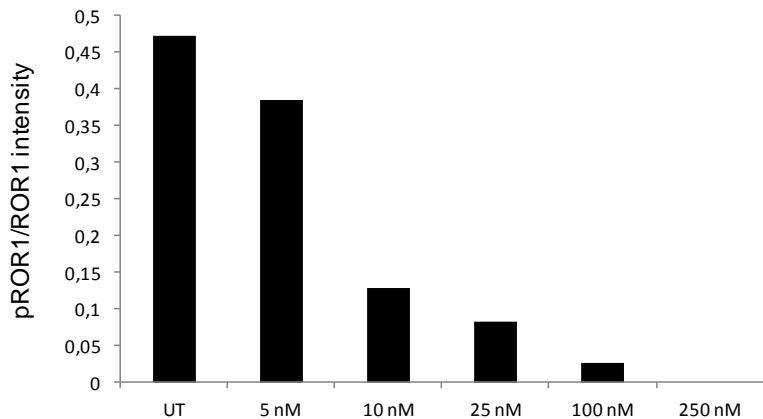
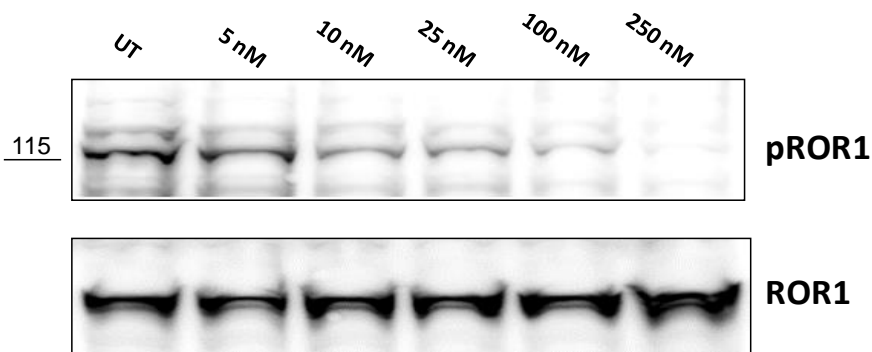


* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

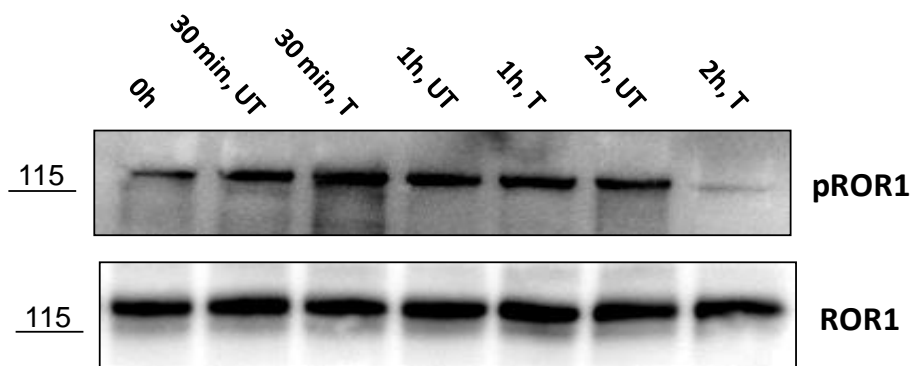
DaneshManesh A, AACR, Abstract, 5-9 April, 2014

ROR1 TKI KAN0439834 and mouse anti-ROR1 CRD mAb induced dephosphorylation of ROR1 in pancreatic carcinoma (PaCa-2) cells

KAN0439834



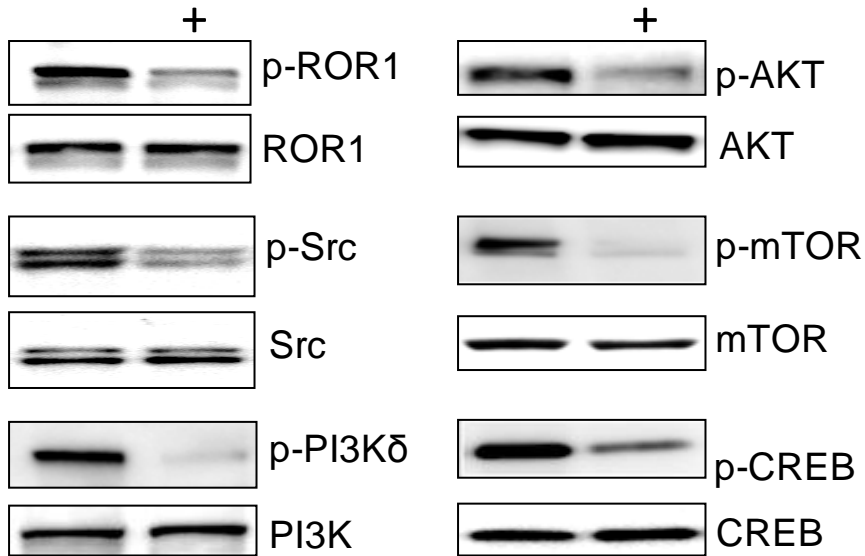
anti-ROR1 CRD MAb



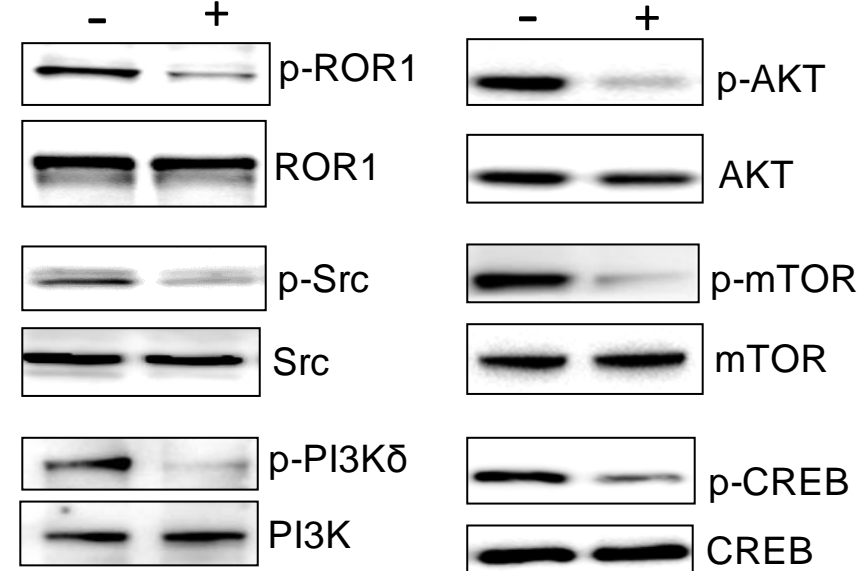
DaneshManesh A, AACR, Abstract, 5-9 April, 2014

ROR1 TKI (KAN0439834) and mouse anti-CRD ROR1 mAbs induced dephosphorylation of ROR1 downstream signaling molecules in pancreatic carcinoma cells (PaCa-2)

KAN0439834



anti-CRD ROR1



From in vitro to in vivo of KAN0439834

Human CLL cells in a NOD SCID mouse xenograft model

Study I:

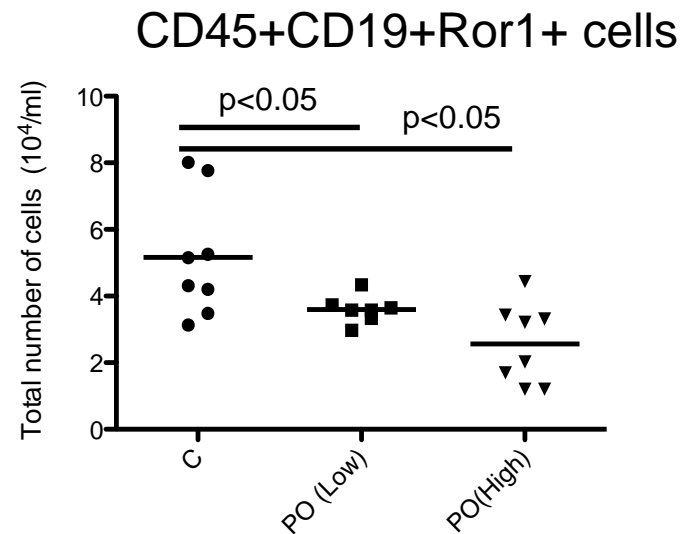
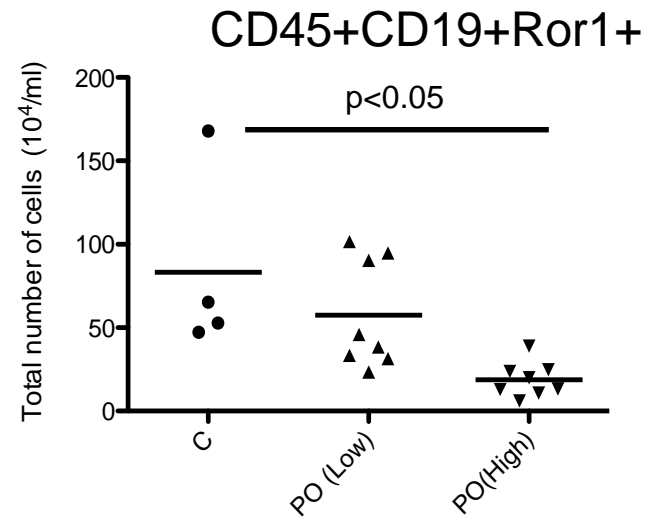
- **Progressive non-17p- CLL donor**

Reduction of CLL cells by 75% on average following 7 day of treatment

Study II:

- **Progressive 17p- CLL donor**

Reduction of CLL cells by 50% on average following 14 day of treatment



Target engagement shown in the lymphoid system/spleen in vivo of KAN0439834

Study I:

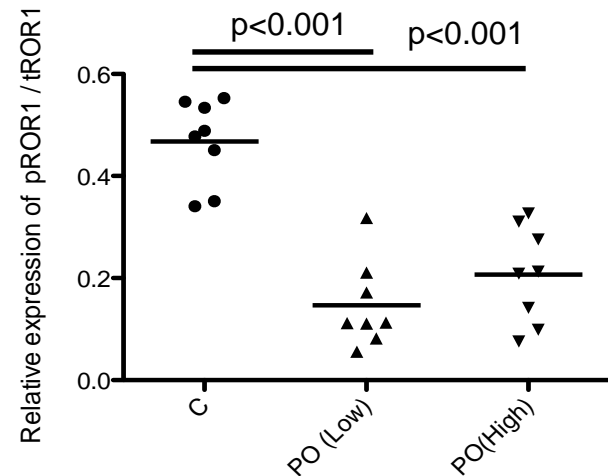
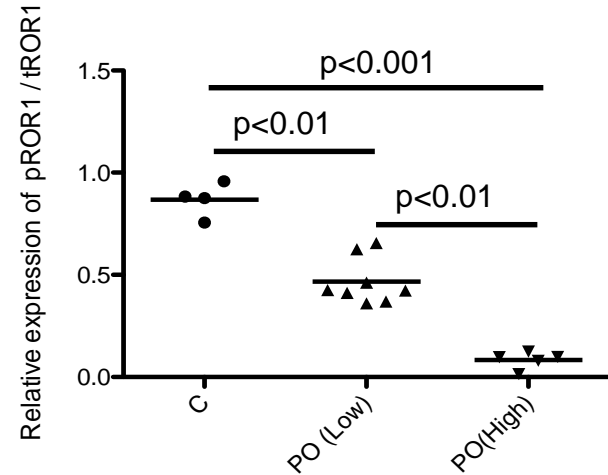
- **Progressive non-17p- CLL donor**

Western blot probed with antibodies against human phosphorylated ROR1

Study II:

- **Progressive 17p- CLL donor**

Western blot probed with antibodies against human phosphorylated ROR1





Other ongoing early clinical and pre-clinical trials using ROR1 as a target

Clinical

- Anti-Ig-3' ROR1 Mab in CLL (Th. Kipps UCLA)
- ROR1-CART in pancreatic carcinoma (D. Maloney, Fred Hutchinson Cancer Research Center, Seattle)
- ROR1-CART in CLL (M. Keating, MD Anderson Cancer Center, Houston, TX)
- ROR1-CD3 bispecific Mabs (MacroGenics Corp.)

Pre-clinical

- ROR1-vaccine inducing an anti-tumor humoral response (Th. Kipps UCLA, H. Mellstedt Karolinska Institute)



Summary

- ROR1 is a unique structure in malignant cells of importance for tumor cell survival.
- Targeting ROR1 by monoclonal antibodies or small molecule TKI induced specific tumor cell death with a high specificity.
- Which is the optimal way to go: large molecules (monoclonal antibodies) or small molecules (TKI)?

Collaborators and Fundings

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Vågberg Jan
Olsson Elisabeth
Norström Carina
Norin Martin



Cancerfonden



European Research Council
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RADIUMHEMETS
FORSKNINGSFONDER



Ragnar Söderbergs stiftelse



Vetenskapsrådet



TORSTEN SÖDERBERGS STIFTELSE