	В					
362 bp	FL	ORF	N19	PVC	WFS	C20
229 bp	FL del 8	1-7	N19	PVC	WFS	
213 bp	Карра	4-11			WFS	C20
157 bp	Beta	ORF			WFS	C20
136 bp	FL del 8-9	1-7	N19	PVC	WFS	
027 bp	FL del 7-9	1-6	N19	PVC	WFS	
954 bp	Pi	ORF	N19	PVC	WFS	C20
412 bp	Gamma	1 -3 stable*	N19	PVC		
263 bp	Gamma del 3	1-2	N19			
206 bp	Gamma del 2-3	1	N19			
186 bp	Gamma del 8	1-3	N19	PVC		
097 bp	Gamma del 2-3, 7	1	N19			
073 bp	Gamma del 2-3, 8	1	N19			
049bp	Gamma del 5-7	1	N19			
009 bp	Phi	ORF	N19			C20
980 bp	Gamma del 2-3, 8-9	1	N19			
952 bp	Delta	ORF	N19			C20
823 bp	Epsilon	ORF	N19	PVC		C20
674 bp	Epsilon del 3	1-2	N19			
617 bp	Eta	ORF				C20
	229 bp 113 bp 57 bp 36 bp 127 bp 154 bp 127 bp 154 bp 127 bp 154 bp 106 bp 106 bp 106 bp 107 bp 173 bp 149bp 109 bp 52 bp 23 bp 74 bp	IAG2 bp   FL     I29 bp   FL del 8     I13 bp   Kappa     57 bp   Beta     36 bp   FL del 8-9     36 bp   FL del 7-9     I12 bp   FL del 7-9     I12 bp   Gamma del 3     I12 bp   Gamma del 3     I12 bp   Gamma del 2-3     I12 bp   Gamma del 2-3, 8     I13 bp   Gamma del 2-3, 8     I14 bp   Gamma del 2-3, 8     I15 bp   Gamma del 2-3, 8     I16 bp   Gamma del 2-3, 8     I17 bp   Gamma del 2-3, 8     I18 bp   Gamma del 2-3, 8     I19 bp   Gamma del 2-3, 8     I19 bp   Gamma del 2-3, 8     I19 bp   Epsilon     <	IAG2 bp   FL   ORF     I29 bp   FL del 8   1-7     I13 bp   Kappa   4-11     57 bp   Beta   ORF     36 bp   FL del 8-9   1-7     36 bp   FL del 7-9   1-6     I12 bp   FL del 7-9   1-6     I12 bp   Gamma   del 3   1-2     I12 bp   Gamma   del 2-3   1     I12 bp   Gamma   del 2-3   1     I13 bp   Gamma   del 2-3   1     I14 bp   Gamma   del 2-3, 7   1     I15 bp   Gamma   del 2-3, 8   1     I17 bp   Gamma   del 2-3, 8   1     I19 bp   Gamma   del 2-3, 8   1     I19 bp   Gamma   del 2-3, 8   1 </td <td>FL   ORF   N19     FL del 8   1-7   N19     FL del 8   1-7   N19     Kappa   4-11   Kappa   4-11     S7 bp   Beta   ORF   Mage     36 bp   FL del 8-9   1-7   N19     FL del 7-9   1-6   N19     Pi   ORF   N19     Gamma   1-3 stable*   N19     Gamma   del 2-3   1   N19     Gamma   del 2-3, 7   1   N19     Gamma   del 2-3, 8   1   N19     Gamma<td>FL   ORF   N19   PVC     I29 bp   FL del 8   1-7   N19   PVC     I13 bp   FL del 8   1-7   N19   PVC     I13 bp   Kappa   4-11  </td><td>FL   ORF   N19   PVC   WFS     129 bp   FL del 8   1-7   N19   PVC   WFS     113 bp   Kappa   4-11   WFS     57 bp   Beta   ORF   WFS     36 bp   FL del 8-9   1-7   N19   PVC   WFS     36 bp   FL del 8-9   1-7   N19   PVC   WFS     36 bp   FL del 7-9   1-6   N19   PVC   WFS     12 bp   Gamma del 3   1-2   N19   PVC   WFS     63 bp   Gamma del 2-3   1   N19   PVC   WFS     66 bp   Gamma del 2-3, 7   1   N19   PVC   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US</td></td>	FL   ORF   N19     FL del 8   1-7   N19     FL del 8   1-7   N19     Kappa   4-11   Kappa   4-11     S7 bp   Beta   ORF   Mage     36 bp   FL del 8-9   1-7   N19     FL del 7-9   1-6   N19     Pi   ORF   N19     Gamma   1-3 stable*   N19     Gamma   del 2-3   1   N19     Gamma   del 2-3, 7   1   N19     Gamma   del 2-3, 8   1   N19     Gamma <td>FL   ORF   N19   PVC     I29 bp   FL del 8   1-7   N19   PVC     I13 bp   FL del 8   1-7   N19   PVC     I13 bp   Kappa   4-11  </td> <td>FL   ORF   N19   PVC   WFS     129 bp   FL del 8   1-7   N19   PVC   WFS     113 bp   Kappa   4-11   WFS     57 bp   Beta   ORF   WFS     36 bp   FL del 8-9   1-7   N19   PVC   WFS     36 bp   FL del 8-9   1-7   N19   PVC   WFS     36 bp   FL del 7-9   1-6   N19   PVC   WFS     12 bp   Gamma del 3   1-2   N19   PVC   WFS     63 bp   Gamma del 2-3   1   N19   PVC   WFS     66 bp   Gamma del 2-3, 7   1   N19   PVC   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US</td>	FL   ORF   N19   PVC     I29 bp   FL del 8   1-7   N19   PVC     I13 bp   FL del 8   1-7   N19   PVC     I13 bp   Kappa   4-11	FL   ORF   N19   PVC   WFS     129 bp   FL del 8   1-7   N19   PVC   WFS     113 bp   Kappa   4-11   WFS     57 bp   Beta   ORF   WFS     36 bp   FL del 8-9   1-7   N19   PVC   WFS     36 bp   FL del 8-9   1-7   N19   PVC   WFS     36 bp   FL del 7-9   1-6   N19   PVC   WFS     12 bp   Gamma del 3   1-2   N19   PVC   WFS     63 bp   Gamma del 2-3   1   N19   PVC   WFS     66 bp   Gamma del 2-3, 7   1   N19   PVC   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US     63 bp   Gamma del 2-3, 8   1   N19   US   US

## BARD1 isoforms in colon cancer.

**A)** BARD1 mRNA isoforms in colon cancer as reported by Sporn et al., 2011 (antibody used: E11 monoclonal undefined regions aa 1-330).

**B)** BARD1 splice variants identified in (**A**) are identical with already known isoforms (Li et al., 2007; Zhang et al., 2011); or forms with or without additional deletions, which results in premature termination of the respective ORFs and presumably unstable proteins. \*Expression confirmed by si-RNA repression.

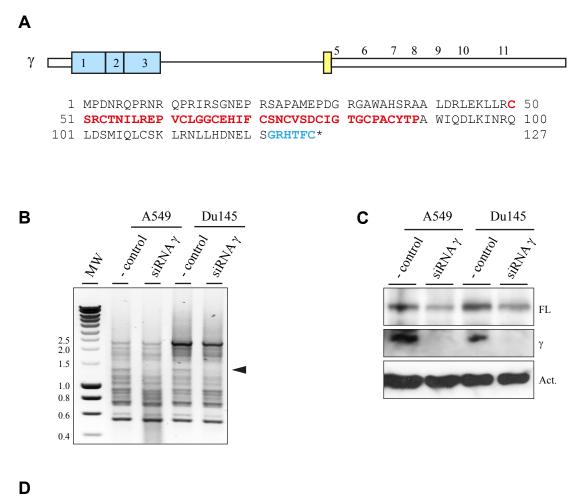
Names of known isoforms are indicated on the left. Known isoforms have an open reading frame (ORF) and are most likely translated. Isoforms with additional deletions are listed as name of known isoform plus indication of deleted exons (del x). These isoforms have shorter ORFs over fewer exons that are indicated. Presence of epitopes recognized by antibodies used in this study (N19, PVC, WFS, C20) is indicated for each isoform.

## **Referenses:**

Li, L., Ryser, S., Dizin, E., Pils, D., Krainer, M., Jefford, C.E., Bertoni, F., Zeillinger, R., and Irminger-Finger, I. (2007) Oncogenic BARD1 isoforms expressed in gynecological cancers. Cancer Res. 67: 11876–11885, doi:10.1158/0008-5472.CAN-07-2370.

Sporn, J.C., Hothorn, T., and Jung, B.H. (2011) BARD1 expression predicts outcome in colon cancer. Clin Cancer Res., doi:10.1158/1078-0432.CCR-11-0263.

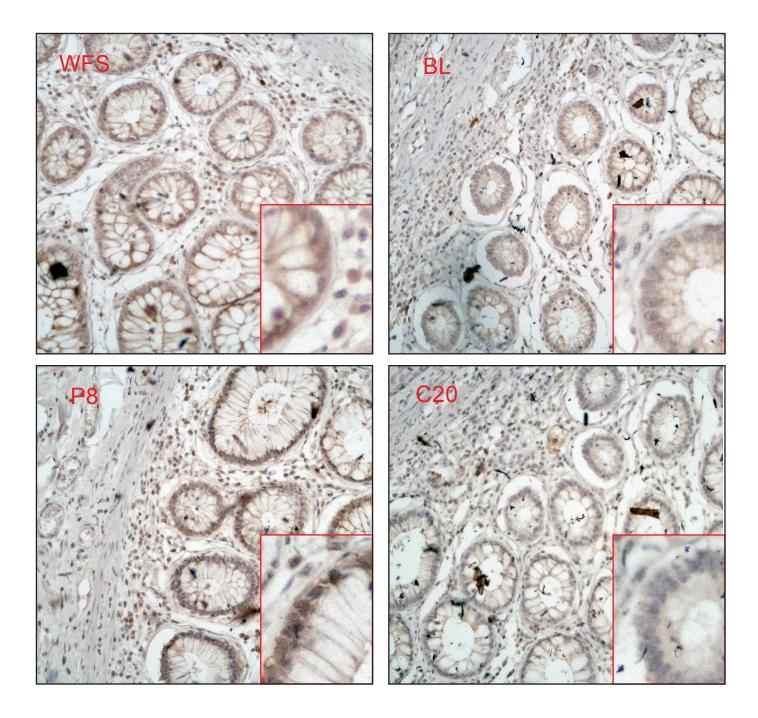
Zhang, Y.-Q., Bianco, A., Malkinson, A.M., Leoni, V.P., Frau, G., Rosa, N.D., André, P.-A., Versace, R., Boulvain, M., Laurent, G.J., Atzuri, L., and Irminger-Finger, I. (2011) BARD1: An independent predictor of survival in non-small cell lung cancer. International Journal of Cancer. Journal International Du Cancer. [Epub ahead of print], doi:10.1002/ijc.26346.



5'- UGA GCU GUC AGG GCG ACA UTT -3' Overhang 5'-3' Sence dTdT Antisense dTdT

## BARD1γ encodes a RING-finger protein and regulates the level of FL BARD1.

**A.** BARD1 gamma mRNA scheme. Non-coding sequences are represented with white bars; alternatively translated sequences are shown as yellow bars. BARD1 $\gamma$  mRNA may encode 14.5kDa polypeptide containing RING-finger domain (shown in red). **B.** Specific BARD1 $\gamma$  siRNA knockdowns BARD1 $\gamma$  mRNA (corresponding RT-PCR fragment is marked with an arrowhead). Non-treated control cells show no change of BARD1 $\gamma$  mRNA level. **C.** The protein corresponding to the expected BARD1 $\gamma$  product is recognized by N-terminus specific N19 antibody and dramatically reduced following BARD1 $\gamma$  siRNA depletion (middle panel). Beta-actin was used as loading control (lower panel). Full length BARD1 was reduced in the cells treated with BARD1 $\gamma$  siRNA (upper panel). This reduction correlates with the reduction of BARD1 $\gamma$  protein level (lower panel). **D.** The sequence of siRNA specific for BARD1 isoform Gamma.



## Comparative staining of colon cancer tumor tissue by antibodies used in the research.

To support the hypothesis that the C20 epitope is present, but not accessible in most isoforms, we have used an antibody against a different sequence in exon 11, as used in a study of lung cancer (Zhang et al., Int J Cancer 2011), for immunohistochemistry of colon cancer samples and compared it to the C20 staining pattern. Similarly, we have performed immunohistochemistry with a commercial antibody BL (exon 4) and compared its staining pattern to that of WFS (exon 4) on a selected number of colon cancer tissue samples.

The staining demonstrates that all four (WFS, BL, P8 and C20) antibodies demonstrate the identical distribution of the signal indicating their specificity. At the same time, C20 staining is weaker that support our hypothesis that C20 epitope is present, but not accessible in most isoforms.