First results of the EIT PACMAN Study: OncoSignal pathway analysis to identify clinically actionable signal transduction pathway activity in a variety of cancer types

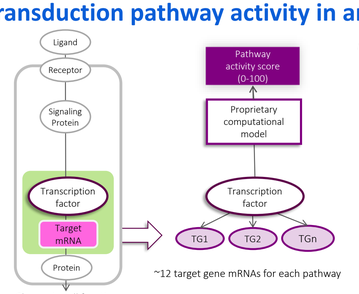
Patricia Martin-Romano; Sigi Neerken; Anja van de Stolpe; Eveline Biezen-Timmermans, Martijn Akse; Maud Ngo-Camus; Claudio Nicotra; A. Eggermont; Paul van de Wiel; Christophe Massard; Fabien Calvo

Institut Gustave Roussy, Paris; Philips Research/Molecular Pathway Dx, Eindhoven, The Netherlands, *Contact:* Anja.van.de.stolpe@philips.com

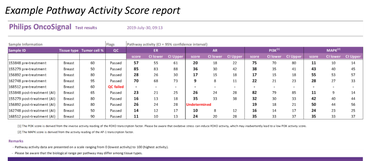
Introduction: Precision medicine may improve outcome of cancer patients by identifying oncogenic alterations and actionable mutations. Yet, DNA sequencing results cannot determine which tumor driving signaling pathways (SP) are functionally active. OncoSignal pathway analysis tests quantitatively measure activity of SP such as *estrogen receptor, androgen receptor, PI3K, MAPK, TGF-β, Notch pathways* on fresh frozen and formalin-fixed paraffin-embedded (FFPE) tissue samples, while mutation analysis provides complementary information related to the (causative) genomic alteration in the SP. Combined information is expected to improve choice of the optimal effective targeted therapy to improve patients' outcome and quality of life as well as to reduce side effects/costs due to unnecessary therapy.

In this study OncoSignal pathway analysis was performed on a series of samples from the Moscato trial (1), with the aim of assessing clinically actionable SP activity.

Methods: Assays for quantitative measurement of signal transduction pathway activity in any cell or tissue type



Example Pathway Activity Score report



Publication list on the Signaling Pathway Analysis assay technology is available at: www.Philips.com/oncosignal

Note: OncoSignal tests are For Research Use Only

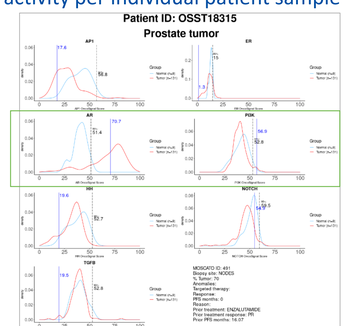
OncoSignal pathway analysis (ER, AR, PI3K, MAPK, HH, Notch, TGF-β) was performed blinded by Molecular Pathway Dx (Philips, Eindhoven) on tumor tissue samples from 5 breast and 31 prostate tumors, all proven to be hard to treat and obtained from the Moscato Study. Results were sent back to Gustave Roussy for clinical annotation and analysis.

For breast and prostate cancer, pathway activity scores were determined for each SP in healthy breast (n=21) and prostate (n=9) tissues (GEO datasets: GSE17951 and GSE10780) and compared with pathway activity scores in cancer tissue samples (multiple GEO datasets: n=131 and n=1686). Increased activity of a pathway in cancer tissue (>95th percentile of healthy tissue pathway activity) was considered as tumor-driver function for the respective pathway and thus clinically actionable. Subsequently, for each individual Moscato sample, alterations were considered as tumor driving pathways if the sample pathway activity score exceeded the 95th percentile of normal (primary) tissue pathway activity.

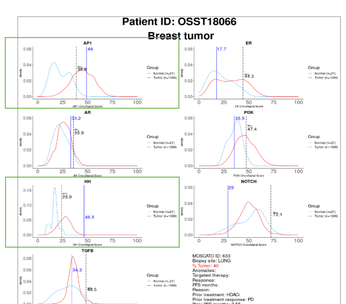
Results

Visualization of abnormal signaling pathway activity per individual patient sample

A



B



Examples of two individual patient samples, (A) prostate cancer, (B) breast cancer. Visualized are for each signaling pathway the pathway activity distribution in healthy tissue (blue line) and in primary cancers originating from this tissue (prostate and breast) (red line); the dotted vertical line indicates the 95% confidence interval of normal pathway activity; the vertical blue line indicates the measured pathway activity in the analyzed sample. If the pathway activity (blue vertical line) is located outside the (right) 95% confidence interval, the pathway activity is considered potentially tumor driving and targetable (green boxed). For each patients this is presented for the ER, AR, PI3K, Hedgehog, MAPK-AP1, Notch, TGFβ pathways.

Identification of actionable tumor driving pathways for each individual patient, breast and prostate cancer

MOSCATO Nr	PRIMARY TUMOR	Cancer genome anomalies	Active pathways	MAPK-AP1	AR	ER	PI3K	Hedgehog	Notch	TGFb
646	breast	TP53 NOTCH2 TSC2	MAPK, HH (NOTCH)	71,1	35,6	20,6	38,7	32,7	69,6	39,1
828	breast	loss TSC1, NOTCH1, CDKN2A	MAPK, ER, PI3K, HH	39,2	28,8	65,8	49,2	30,6	32,1	30,5
679	breast	NOTCH2 rearr; TP53	HH, PI3K, MAPK	42,9	20,1	15,6	58,7	31,1	53,7	25,7
633	breast		MAPK,HH	49	33,2	17,7	35,5	46,5	29	34,3
948	breast	ALKrear	MAPK, HH	54,7	28,9	31,5	43,8	30,5	55,8	34,9
241	prostate	AMPLIFICATION_RECEPTEUR_AUX_ANDROGENES	AR, ER	20,8	72,1	16	40,8	29,8	55,2	27,8
260	prostate	MUTATION_PIK3CA	AR	26,7	80,8	6,9	48	40	57,1	25,3
522	prostate	AMPLIFICATION AR/AMPLIFICATION NOTCH2	AR	30,1	75,4	1,9	41,5	29,5	53,8	22,3
215	prostate	AMPLIFICATION_AR	AR	24,4	79	11,7	25,8	30,6	46,8	16,2
618	prostate	MUTATION PI3K/AMPLIFICATION FGF4/MUTATION FGF3/MUTATION FGF19/MUTATION NOTCH4	MAPK, AR	58,1	53,5	5,1	34,2	35,8	45	33,5
689	prostate	DELETION_RB1	AR	21,4	68,7	3,8	44,3	25,5	49,9	21,5
181	prostate	MUTATION_PIK3CA								
163	prostate		ER,HH	32,3	24,9	24,1	43	36,9	30,4	21,6
555	prostate		AR,ER,HH	45,7	58,9	11,7	47,9	55,2	54,5	26,9
202	prostate	DELETION RB1	ER, PI3K	30,3	20,7	17,8	54,6	47,5	33,5	27,2
575	prostate	MUTATION TSC2	AR,ER	20	53,2	15,2	47,3	22,9	22,6	11,6
978	prostate	MUTATION_BRCA2	PI3K	19,2	49,7	5,1	59,8	35,5	30,3	29
1092	prostate		AR, PI3K	38,7	74,8	3,9	60	25,1	44,6	15,2
975	prostate		AR	40	62,5	7,4	44,8	29,4	56,1	28,5
1025	prostate		AR	12,5	78,7	11,7	39,7	22,1	38,4	20,7
1006	prostate		AR, PI3K, NOTCH	26	68	9,3	57,7	36,3	63	32,3
414	prostate	AMPLIFICATION MDM2								
486	prostate									
623	prostate			47,6	28,7	9,6	38,7	15,6	16,6	26
598	prostate		ER	51,3	29,2	16,5	42,9	32,5	29,6	33,7
277	prostate		AR	33,4	78	12,9	44,4	25,3	43,9	12,4
461	prostate		AR	26,1	59,7	9	40,9	35	44,7	18,1
222	prostate		AR	19,5	62	3	47,9	29,1	48,1	16
359	prostate	AMPLIFICATION_RECEPTEUR_AUX_ANDROGENES/ AMPLIFICATION_PIK3CA	AR	9,8	72,4	4,3	45,8	23,5	45,1	11,8
518	prostate	AMPLIFICATION PI3K	AR, NOTCH	52,3	55,7	9,6	44,3	27,8	60,4	52,7
674	prostate		AR, NOTCH	14,8	66,9	4,1	39,4	16,1	61	16,1
491	prostate		AR, PI3K	17,6	70,7	1,3	56,9	19,6	54,9	19,5
613	prostate		AR	25,3	82,2	6,6	45,9	28,1	47,4	14,9
497	prostate		AR	14,7	65,6	10,2	41	18,4	46,9	20,8
869	prostate		AR	10	65	4,2	32,6	17,4	37,2	8,8
262	prostate		AR	11,9	91,8	13,8	41,9	16,4	59	9
698	prostate		AR	15,9	81,6	5,1	35,8	21,8	54,4	13,9
217	prostate		AR	7,2	78,8	1	49,9	23,4	17,4	11,7
484	prostate	AMPLIFICATION MDM2	AR	21,3	88,9	8,8	45	18,3	46,4	25,6
218	prostate	MUTATION_PIK3CA								

Columns show from left to right:
- sample identification number,
- primary tumor type,
- genome mutations,
- identified actionable pathway activities (black lined boxes),
- pathway activity in agreement with genomic mutations;
- pathway activity score for MAPK-AP1; AR, ER, PI3K, Hedgehog, Notch and TGFβ pathways (normalized scale 0-100).

Boxed pathway activity scores indicate that activity exceeds the 95the percentile of the activity in normal tissue (respectively for breast and prostate).

Identification of mutations as a cause for activation of a tumor driving pathway and prediction of response to targeted drugs, breast and prostate cancer

MOSCATO	Primary Tumor	Metastatic Tumor	TUMOR %	Cancer genome anormalities	Treatment	Treated pathway	PFS2/ PFS1	Response	Active Pathways	MAPK-AP1	AR	ER	PI3K	Hedgehog	Notch	TGFb
646	Breast	HEAD AND NECK	40	TP53 NOTCH2 TSC2	AFINITOR	PI3K	1,57	responder	MAPK, HH, Notch	71,1	35,6	20,6	38,7	32,7	69,6	39,1
679	Breast	CUT / SOUS CUT	15	NOTCH2 rearr; TP53	NOTCHI	Notch	0,325	non-responder	HH, PI3K, MAPK	42,9	20,1	15,6	58,7	31,1	53,7	25,7
828	Breast	LIVER	50	loss TSC1, NOTCH1, CDKN2A	AFINITOR	PI3K	0,562	non-responder	ER, HH, MAPK, PI3K	39,2	28,8	65,8	49,2	30,6	32,1	30,5
215	Prostate	NODES	80	AMPLIFICATION_AR	ENZALUTAMINE	AR	0,675	non-responder	AR	24,4	79	11,7	25,8	30,6	46,8	16,2
241	Prostate	LIVER	40	AMPLIFICATION_RECEPTEUR_AUX_ANDROGENES	ABIRATERONE	AR	0,798	non-responder	AR, ER	20,8	72,1	16	40,8	29,8	55,2	27,8
260	Prostate	NODES	60	MUTATION_PIK3CA	CETUXIMAB +TEMSIROLOMUS	MAPK, PI3K	0,111	non-responder	AR	26,7	80,80	6,90	48,00	40,00	57,1	25,3
522	Prostate	NODES	70	AMPLIFICATION AR/AMPLIFICATION NOTCH2	ENZALUTAMINE	AR		NOT evaluable	AR	30,1	75,4	1,9	41,5	29,5	53,8	22,3
618	Prostate	LIVER	40	MUTATION PI3K/AMPLIFICATION FGF4/MUTATION FGF3/MUTATION FGF19/MUTATION NOTCH4	NOTCH INHIBITOR	Notch		NOT evaluable	MAPK, AR	58,1	53,5	5,1	34,2	35,8	45	33,5
689	Prostate	NODES	60	DELETION_RB1	NOTCH INHIBITOR	Notch	3,584	responder	AR	21,4	68,7	3,8	44,3	25,5	49,9	21,5

Columns show individual patient information for patients treated with a targeted drug and pathway activiies. Calculation of responder status was according to MOSCATO (response was calculated as *time to progression* divided by *time to progression on the previous given therapy)*; identified actionable pathways are boxed. NE=non evaluable

Conclusion: OncoSignal analysis of signal transduction pathway activities in cancer tissue samples enabled identification of potentially clinically actionable (targeted drugs) signaling pathway activity in 97-100% of analyzed breast and prostate cancer samples.

The PACMAN study is supported by EIT Health

EIT Health is supported by the EIT, a body of the European Union