Phase I study of single-agent WNT974, a first-in-class Porcupine inhibitor, in patients with advanced solid tumours

Supplementary information

Supplementary Table 1. Additional information for patients in the expansion phase.

Primary diagnosis	Baseline genetic alterations related to study eligibility and the Wnt pathway *(allelic frequency[a])*					Best percentage change in sum of target lesion diameters (%)
	BRAF V600E	*RNF43* mutation	*RSPO* fusion	Other selected Wnt pathway alteration[b]		
				Mutation	Other alteration	
CRC[c]	V600E	W416X	–	–	–	–11.54
Neuroendocrine carcinoma[c]	–	R145	–	–	–	41.67
SCLC[d]	–	E187L *(28.0)*	–	–	–	4.44
CRC[d]	V600E *(18.0)*	L163fs*2 *(27.0)*	–	–	–	15.38
Pancreatic cancer[d]	–	H427fs*16 *(45.0)*	–	–	–	6.08
CRC[d]	WT	D140H *(25.0)*	–	*APC* E1151* *(27.0)*	–	36.05
CRC[d]	WT	Y177C	–	–	–	18.73
CRC[d]	V600E *(19.0)*	–	E2 gene fusion	–	–	5.43
CRC[d]	WT	R114W *(26.6)*	–	*APC* G1312* *(78.8)*	–	81.67
Chondro-sarcoma[e]	–	–	–	*ZNRF3* S457del *(34.4)*	–	16.10
Head and neck cancer[d]	–	–	–	*ZNRF3* V156M *(49.0)*	–	UNK
CRC[d]	V600E *(16.0)*	WT	–	*APC* R213* *(14.1)* *APC* Q1480* *(20.2)* *ZNRF3* N29K *(15.4)*	–	6.38
Pancreatic cancer[d]	–	L418M	–	*APC* R2237* *(34.0)*	–	–4.76
Pancreatic cancer[d]	–	R343H	–	–	–	64.81
Ovarian[d]	–	P686R	–	–	–	2.56
Pancreatic cancer[c]	–	P231L R343H L418M	–	–	–	37.50
Endometrial cancer[d]	–		–	*CTNNB1* D32Y *(36.7)*	–	64.84
CRC[d]	V600E *(12.0)*	L311fs*10 8 *(10.0)*	–	*ZNRF3* S178fs*25 *(10.0)*	–	UNK
Appendiceal goblet cell carcinoma[d]	–	S41* Y450fs*32 *(22.0)*	–	–	–	–26.81

Gastric cancer[c]	–	Exon 4	–	–	–	–5.34
Sarcoma[c]	–	V107I	–	–	–	6.06
Cholangio-carcinoma[c]	–	G659fs*41	–	*APC* K1616	–	34.04
Cholangio-carcinoma[c]	–	L302M	–	–	–	UNK
Pancreatic cancer[e]	–	R371* *(34.0)*	–	–	–	23.46
Pancreatic cancer[e]	–	–	–	–	LRP6 [amplification]	37.78
CRC[d]	WT	R531H *(10.0)*	–	*APC* S1465fs*3 *(8.0)* *APC* T1208fs*57 *(10.0)* *LRP6* P1059S *(8.0)* *WISP3* R339K *(9.0)* *ZNRF3* P79L *(9.0)*	RNF43 *(11.0)* [splice site alteration]	30.0
Pancreatic cancer[d]	–	R117G *(43.0)*	–	–	–	4.96
Endometrial cancer[c]	–	G659fs*41	–	–	–	10.61

Patients with some reduction in target lesion size are highlighted in blue.

[a]Allelic frequency provided where available.

[b]Selected genes (*RNF43*; *RSPO*; *ZNRF*; *APC*; *CTNNB1*; *LRP6*; *WISP3*).

[c]Genetic alterations as determined by local testing.

[d]Genetic alterations as determined by local and central testing.

[e]Genetic alterations as determined by central (next-generation sequencing) testing.

Abbreviations: CRC, colorectal cancer; del, deletion; fs, frameshift; RNF43, ring finger protein 43; RSPO, R-spondin; SCLC, small cell lung cancer; UNK, unknown; WT, wild type.

Supplementary Table 2. Pharmacokinetic parameters of WNT974 in patients following oral administration of WNT974.

	WNT974 5 mg QD	**WNT974 7.5 mg QD**	**WNT974 10 mg QD**	**WNT974 15 mg QD**	**WNT974 20 mg QD**	**WNT974 22.5 mg QD**	**WNT974 30 mg QD**	**WNT974 30 mg 4/7 QD**	**WNT974 45 mg 4/7 QD**	**WNT974 5 mg BID**
C1D1										
n	6	6	38	10	10	6	5	4	3	5
C_{max} (ng/mL)	33.0 (26.4)	48.1 (47.4)	72.6 (51.0)	90.9 (29.0)	144 (24.6)	178 (40.2)	143 (64.5)	208 (46.1)	473 (88.6)	25.6 (65.2)
T_{max} (h)	1.98 (1.0–4.0)	2.00 (2.0–4.0)	1.75 (0.5–4.0)	2.00 (1.0–3.1)	2.02 (1.0–4.1)	1.00 (0.5–3.0)	1.00 (0.5–3.1)	2.50 (2.0–3.0)	1.00 (0.5–6.0)	3.00 (2.0–3.2)
AUC_{tau} (h·ng/mL)	245 (9.8)	290 (43.4)	434 (40.1)	567 (27.2)	1,083 (27.2)	965 (33.8)	928 (63.6)	1,681 (60.6)	3,432 (13.9)	103 (64.1)
$T_{1/2}$	4.83 (56.1)	5.14 (42.8)	5.67 (23.7)	6.28 (19.3)	7.60 (51.0)	6.50 (33.5)	7.57 (59.8)	5.42 (26.6)	5.34 (32.2)	10.19 (ND)
C1D15										
n	4	5	30	8	7	4	4	4	3	4
C_{max} (ng/mL)	33.4 (52.9)	61.4 (55.1)	90.4 (44.0)	128 (46.5)	197 (38.2)	191 (56.0)	251 (64.6)	260 (52.5)	373 (51.4)	31.3 (94.5)
T_{max} (h)	2.98 (0.5–3.0)	1.00 (0.5–4.1)	1.98 (0.5–4.0)	2.50 (0.5–3.0)	2.00 (0.5–3.0)	1.00 (1.0–1.1)	1.00 (1.0–1.0)	2.50 (2.0–6.1)	4.00 (1.0–6.0)	3.00 (1.0–4.0)
AUC_{tau} (h·ng/mL)	294 (29.6)	443 (8.92)	627 (37.7)	793 (36.0)	1,634 (35.8)	1,125 (45.2)	1,879 (40.1)	2,082 (71.7)	3,693 (32.1)	141 (88.3)
$T_{1/2}$	6.82 (39.1)	6.82 (15.1)	6.33 (20.7)	6.18 (14.4)	7.79 (29.01)	9.52 (17.0)	6.29 (19.9)	6.31 (12.7)	5.35 (15.8)	4.84 (20.5)
C_{min} (ng/mL)	2.40 (56.6)	3.91 (66.4)	4.67 (59.4)	4.92 (42.5)	16.4 (162)	10.5 (107)	17.2 (68.0)	NA	NA	4.71 (135)
Racc	1.16 (21.0)	1.31 (26.2)	1.50 (34.5)	1.50 (35.7)	1.58 (52.6)	1.18 (32.9)	1.67 (16.8)	NA	NA	1.61 (27.6)

Geometric mean (geometric CV%) provided, except for T_{max} which is median (range).

Dose escalation and expansion phases; data cutoff: 2 March 2017.

Abbreviations: 4/7 QD, drug administered 4 days on, 3 days off; AUC_{tau}, area under the curve for the dosing interval; BID, twice daily; C, Cycle; C_{max}, maximum concentration; C_{min}, minimum observed plasma concentration during a dosing interval; D, day; NA, not applicable; ND, no data available; QD, once daily; Racc, accumulation ratio, calculated as AUC_{tau}, Day 15/AUC_{tau}, Day 1; $T_{1/2}$, terminal elimination half-life; T_{max}, time of C_{max}.

Supplementary Table 3. Pharmacokinetic parameters of LHA333 in patients following oral administration of WNT974.

	WNT974 5 mg QD	**WNT974 7.5 mg QD**	**WNT974 10 mg QD**	**WNT974 15 mg QD**	**WNT974 20 mg QD**	**WNT974 22.5 mg QD**	**WNT974 30 mg QD**	**WNT974 30 mg 4/7 QD**	**WNT974 45 mg 4/7 QD**	**WNT974 5 mg BID**
C1D1										
n	6	6	38	10	10	6	5	4	3	5
C_{max} (ng/mL)	5.61 (35.1)	12.0 (43.8)	19.1 (64.1)	26.9 (63.6)	37.5 (76.7)	48.9 (47.4)	41.2 (84.3)	72.6 (36.5)	129 (179.8)	6.88 (71.2)
T_{max} (h)	3.00 (2.0–6.0)	3.07 (2.0–6.0)	2.03 (0.5–8.0)	3.00 (2.0–8.0)	3.00 (1.0–8.0)	2.00 (0.6–3.1)	1.00 (1.0–3.1)	3.00 (2.0–4.0)	2.00 (0.5–8.0)	3.00 (2.0–6.0)
AUC_{tau} (h·ng/mL)	54.2 (55.4)	149 (33.2)	208 (50.9)	333 (46.3)	426 (37.3)	457 (28.5)	458 (68.9)	802 (33.4)	1,907 (1.20)	36.5 (81.3)
M/P ratio	ND	0.52 (88.9)	0.50 (34.0)	0.58 (55.8)	0.41 (57.2)	0.47 (25.9)	0.49 (29.7)	0.47 (41.6)	0.55 (14.2)	ND
C1D15										
n	4	5	30	8	7	4	4	4	3	4
C_{max} (ng/mL)	6.17 (66.1)	9.39 (68.1)	19.4 (58.2)	29.7 (42.3)	51.6 (57.4)	34.6 (75.7)	46.4 (104.0)	62.9 (47.4)	87.2 (80.6)	7.04 (52.8)
T_{max} (h)	3.50 (1.0–6.0)	1.00 (0.5–8.0)	2.54 (0.5–7.5)	3.00 (0.5–4.1)	1.03 (0.5–6.1)	1.00 (1.0–1.1)	1.00 (1.0–0.8)	2.50 (2.0–8.0)	4.00 (3.0–6.0)	9.00 (1.0–12.0)
AUC_{tau} (h·ng/mL)	95.7 (84.4)	119 (18.4)	222 (53.7)	345 (42.2)	657 (35.4)	367 (49.3)	644 (78.0)	810 (44.0)	1,527 (59.2)	34.1 (124.2)
M/P ratio	0.25 (86.7)	0.26 (10.5)	0.33 (49.2)	0.42 (53.3)	0.39 (55.0)	0.31 (52.6)	0.31 (34.5)	NA	NA	0.23 (32.5)
Racc	1.59 (20.6)	0.86 (37.2)	1.16 (34.5)	1.09 (35.7)	1.30 (26.2)	0.78 (56.2)	0.98 (48.6)	NA	NA	1.17 (42.8)

Geometric mean (geometric CV%) provided, except for T_{max} which is median (range).

Dose escalation and expansion phases; data cutoff: March 2, 2017.

Abbreviations: 4/7 QD, drug administered 4 days on, 3 days off; AUC_{tau}, area under the curve for the dosing interval; BID, twice daily; C, Cycle; C_{max}, maximum concentration; D, Day;
M/P ratio: metabolite-to-parent ratio, calculated as AUC_{inf}, LHA333/AUC_{inf}, WNT974 for Day 1 or AUC_{tau}, LHA333/AUC_{tau}, WNT974 for Day 15, where the AUC_{tau} values are corrected by molecular weight; NA, not applicable; ND, no data available; QD, once daily;
Racc, ratio of accumulation, calculated as AUC_{tau}, Day 15/AUC_{tau}, Day 1;
$T_{1/2}$, terminal elimination half-life; T_{max}, time of C_{max}.

Supplementary Table 4. Adverse events (Grade ≥3, occurring in ≥5% of patients, regardless of study drug relationship) by treatment group.

	WNT974 5 mg QD *n*=6	**WNT974 7.5 mg QD *n*=6**	**WNT974 10 mg QD *n*=38**	**WNT974 15 mg QD *n*=11**	**WNT974 20 mg QD *n*=10**	**WNT974 22.5 mg QD *n*=6**	**WNT974 30 mg QD *n*=5**	**WNT974 30 mg 4/7 QD *n*=4**	**WNT974 45 mg 4/7 QD *n*=3**	**WNT974 5 mg BID *n*=5**	**All patients *N*=94**
Total *n* (%)	3 (50.0)	5 (83.3)	26 (68.4)	8 (72.7)	9 (90.0)	5 (83.3)	4 (80.0)	1 (25.0)	3 (100)	2 (40.0)	66 (70.2)
Asthenia	0	1 (16.7)	3 (7.9)	1 (9.1)	1 (10.0)	1 (16.7)	0	1 (25.0)	0	0	8 (8.5)
Fatigue	0	0	4 (10.5)	1 (9.1)	1 (10.0)	0	1 (20.0)	1 (25.0)	0	0	8 (8.5)
Dehydration	0	0	2 (5.3)	0	0	1 (16.7)	1 (20.0)	0	3 (100)	0	7 (7.4)
Dyspnoea	1 (16.7)	1 (16.7)	2 (5.3)	1 (9.1)	0	1 (16.7)	0	1 (25.0)	0	0	7 (7.4)
Vomiting	0	0	3 (7.9)	1 (9.1)	1 (10.0)	1 (16.7)	0	0	1 (33.3)	0	7 (7.4)
Anaemia	0	1 (16.7)	3 (7.9)	0	0	1 (16.7)	0	0	0	1 (20.0)	6 (6.4)
Nausea	0	0	3 (7.9)	0	0	2 (33.3)	0	0	1 (33.3)	0	6 (6.4)
Abdominal pain	1 (16.7)	0	3 (7.9)	0	1 (10.0)	0	0	0	0	0	5 (5.3)
Blood ALP increased	0	1 (16.7)	3 (7.9)	1 (9.1)	0	0	0	0	0	0	5 (5.3)
Hyper-calcaemia	0	0	3 (7.9)	0	0	2 (33.3)	0	0	0	0	5 (5.3)

Data cutoff: 2 March 2017.

Abbreviations: 4/7 QD, drug administered 4 days on, 3 days off; ALP, alkaline phosphatase, BID, twice daily; QD, once daily.

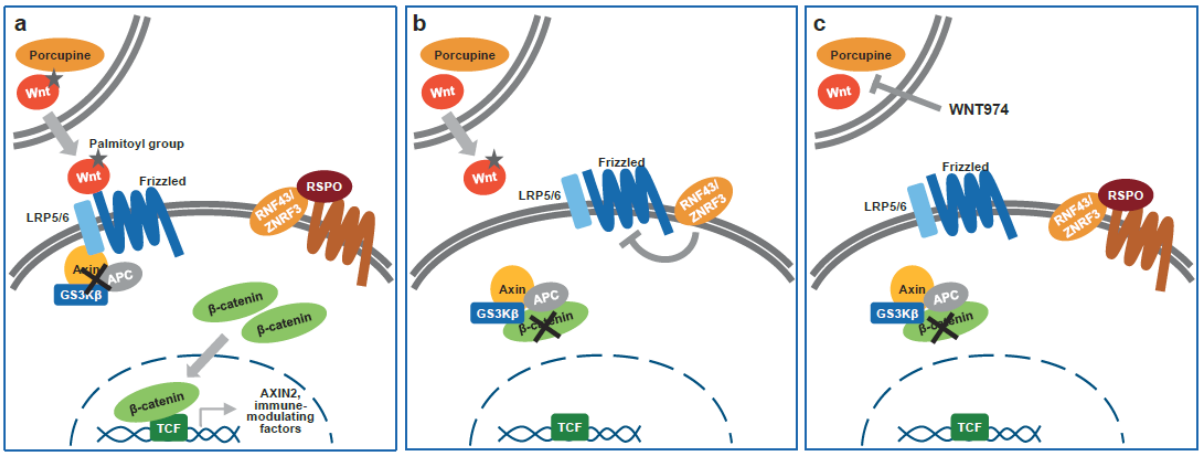
Supplementary Table 5. Best overall response by treatment group.

n (%)	WNT974 5 mg QD *n*=6	WNT974 7.5 mg QD *n*=6	WNT974 10 mg QD *n*=38	WNT974 15 mg QD *n*=11	WNT974 20 mg QD *n*=10	WNT974 22.5 mg QD *n*=6	WNT974 30 mg QD *n*=5	WNT974 30 mg 4/7 QD *n*=4	WNT974 45 mg 4/7 QD *n*=3	WNT974 5 mg BID *n*=5	All patients *N*=94
Complete response	0	0	0	0	0	0	0	0	0	0	0
Partial response	0	0	0	0	0	0	0	0	0	0	0
Stable disease	0	1 (16.7)	11 (28.9)	1 (9.1)	1 (10.0)	0	1 (20.0)	0	0	0	15 (16.0)
Progressive disease	3 (50.0)	5 (83.3)	21 (55.3)	6 (54.5)	7 (70.0)	5 (83.3)	2 (40.0)	2 (50.0)	2 (66.7)	3 (60.0)	56 (59.6)
Non-CR/ non-PD	0	0	0	1 (9.1)	0	0	0	0	0	0	1 (1.1)
Unknown[a]	3 (50.0)	0	6 (15.8)	3 (27.3)	2 (20.0)	1 (16.7)	2 (40.0)	2 (50.0)	1 (33.3)	2 (40.0)	22 (23.4)

Data cutoff: 2 March 2017.

[a]Best overall response was unknown in 22 patients, due to discontinuation prior to post-baseline assessment in 16 patients, or single assessment not qualifying for a RECIST v1.1 category in 6 patients (SD prior to 6 weeks [n=5] and PD after 12 weeks [n=1]).

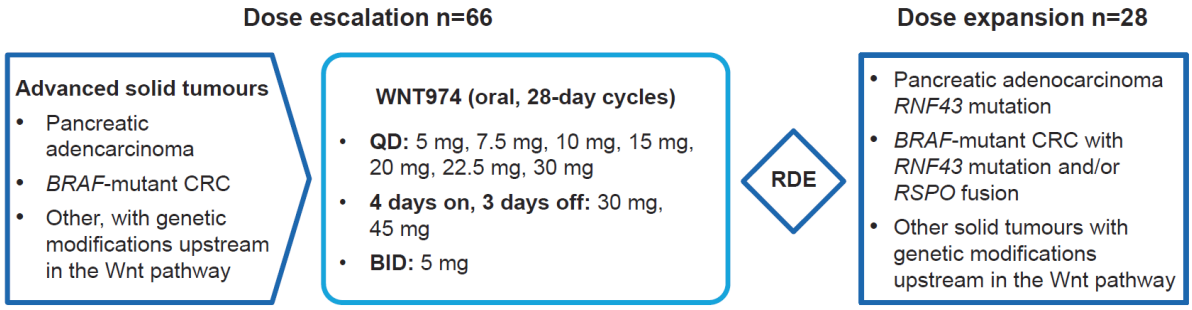
Abbreviations: 4/7 QD, drug administered 4 days on, 3 days off; BID, twice daily; CR, complete response; PD, progressive disease; QD, once daily; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.



Supplementary Fig. 1. The canonical Wnt/β-catenin signalling pathway.

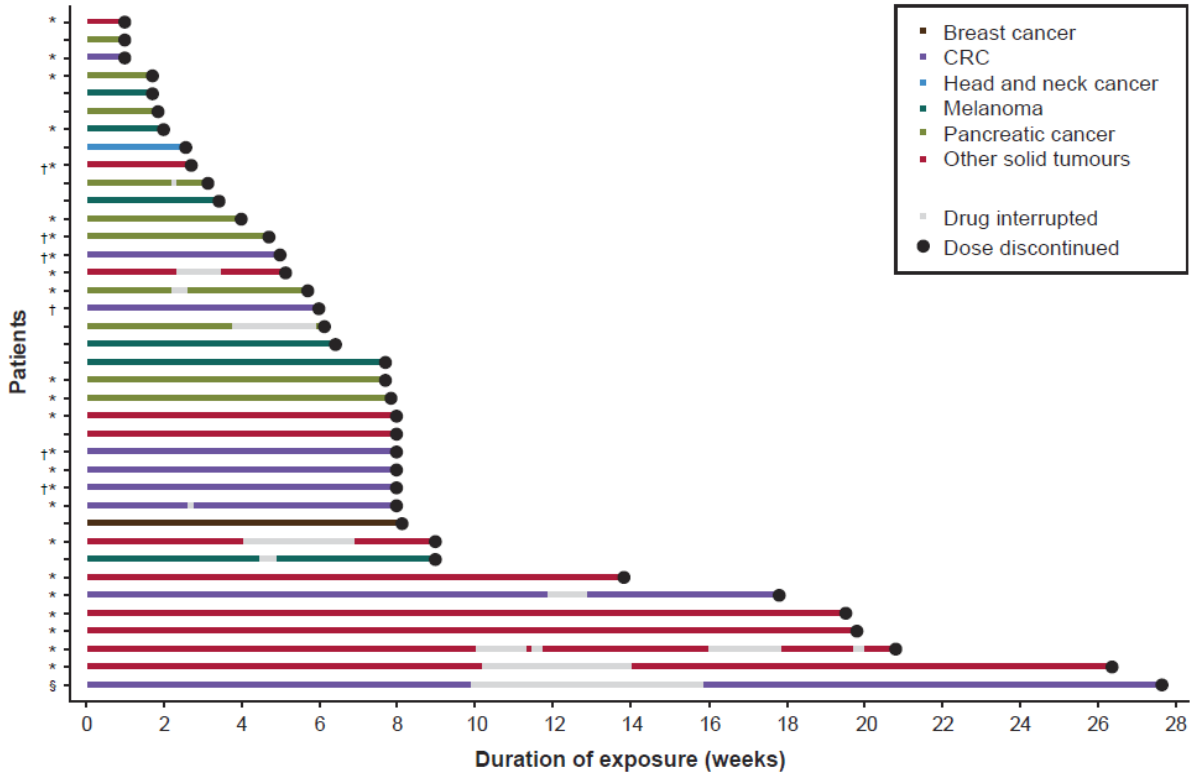
a) Palmitoylated Wnt binds co-receptors, LRP5/6 and Frizzled, leading to inactivation of the β-catenin destruction complex. RSPO inhibits RNF43/ZNRF3, increasing levels of the Wnt receptor complex on the cell surface. β-catenin accumulates in the cytoplasm, translocates to the nucleus, and subsequently stimulates transcription of specific genes, including AXIN2. **b)** When not suppressed by RSPO, RNF43/ZNRF3 targets the Wnt receptor complex for degradation, inhibiting Wnt signalling. In the absence of Wnt ligand binding to the receptor complex, the β-catenin destruction complex is active in the cytoplasm and mediates degradation of β-catenin. **c)** In the presence of WNT974, palmitoylation of Wnt ligands is inhibited, preventing secretion.

Abbreviations: APC, adenomatous polyposis coli; LRP5/6, low-density lipoprotein receptor-related protein 5/6; RNF43, ring finger protein 43; RSPO, R-spondin; ZNRF3, zinc and ring finger protein 3.



Supplementary Fig. 2. Study design.

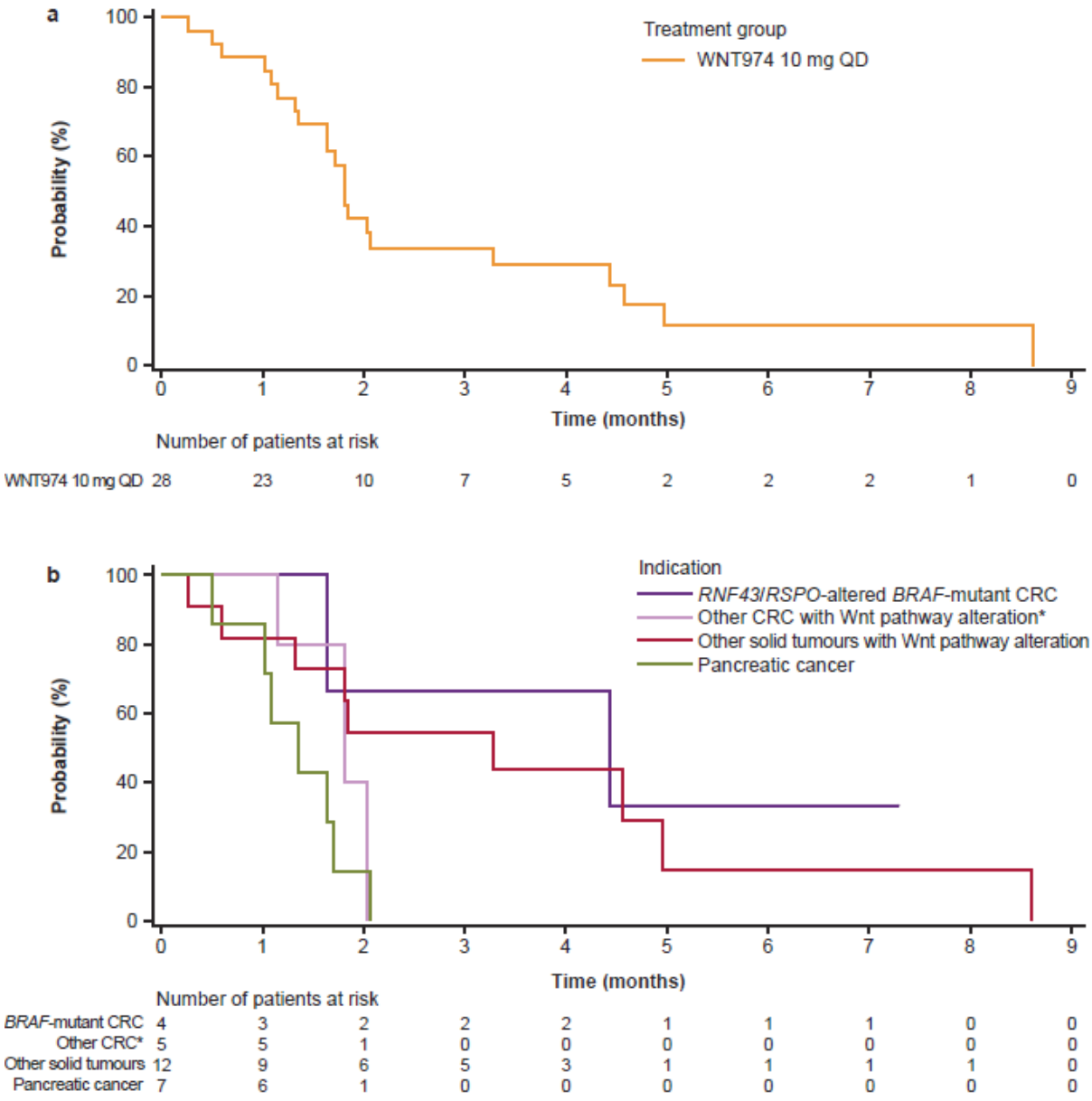
Abbreviations: BID, twice daily; CRC, colorectal cancer; QD, once daily; RDE, recommended dose for expansion; RNF43, ring finger protein 43; RSPO, R-spondin.



Supplementary Fig. 3. Duration of exposure to WNT974 in patients receiving 10 mg QD.

**RNF43* alteration; †*APC* alteration; §*RSPO* fusion. Data cutoff: 2 March 2017.

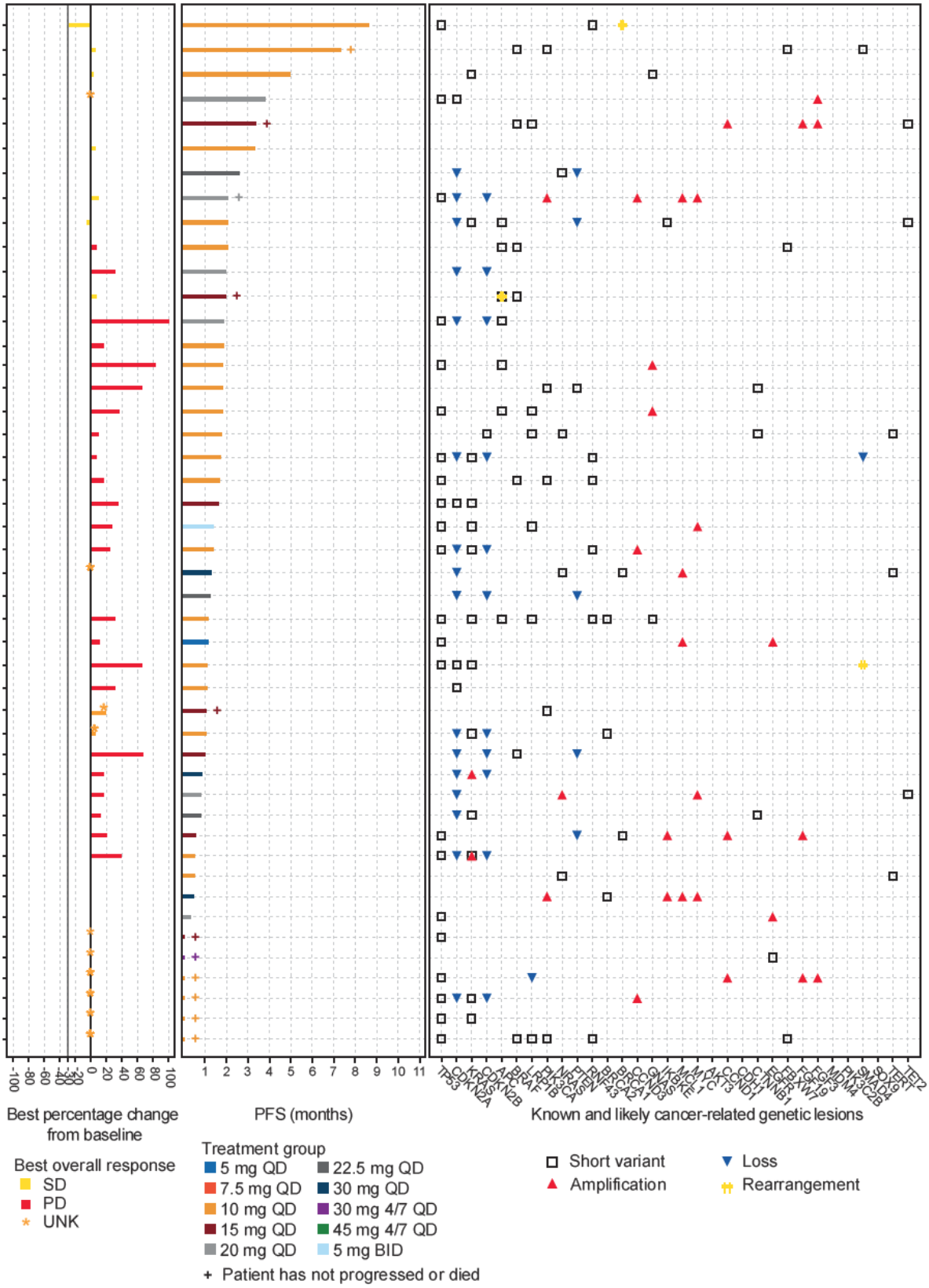
Abbreviations: APC, adenomatous polyposis coli; CRC, colorectal cancer; QD, once daily; RNF43, ring finger protein 43; RSPO, R-spondin.



Supplementary Fig. 4. Kaplan–Meier plots of progression-free survival (expansion part).

Progression-free survival probability is shown for **a)** all patients in the expansion part of the study and **b)** patients in the expansion part of the study, separated by indication. *Including *BRAF*-wild-type CRC with *RNF43* mutation. Data cutoff: 2 March 2017.

Abbreviations: CRC, colorectal cancer; QD, once daily; RNF43, ring finger protein 43; RSPO, R-spondin.


-100
-80
-60
-40
-20
0
20
40
60
80
100
Best percentage change from baseline
Best overall response
SD
PD
UNK
1
2
3
4
5
6
7
8
9
10
11
PFS (months)
Treatment group
5 mg QD
7.5 mg QD
10 mg QD
15 mg QD
20 mg QD
22.5 mg QD
30 mg QD
30 mg 4/7 QD
45 mg 4/7 QD
5 mg BID
Patient has not progressed or died
TP53
CDKN2A
KRAS
CDKN2B
ARID1A
BRAF
PIK3CA
PTEN
RNF43
BRCA2
CCND3
NTRK3
MYC
MCL1
DAXX
CCND1
CTNNB1
FGFR3
FGF19
MDM4
SMAD4
SOX9
TERT
ERBB2
Known and likely cancer-related genetic lesions
Short variant
Amplification
Loss
Rearrangement

Supplementary Fig. 5. Select genetic alterations ordered by best percentage change from baseline. Best percentage change from baseline and PFS are shown for each patient, alongside alterations in selected genes of known and likely functional significance to cancer, observed in more than 2 patients. Data cutoff: 2 March 2017.

Abbreviations: 4/7 QD, drug administered 4 days on, 3 days off; BID, twice daily; PD, progressive disease; PFS, progression-free survival; QD, once daily; SD, stable disease; UNK, unknown.