Phase I expansion and pharmacodynamic study of the oral MEK inhibitor RO4987655 (CH4987655) in selected advanced cancer patients with RAS-RAF mutations

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Translational relevance

This multicenter phase I expansion (part 2) study has demonstrated that the MEK inhibitor RO4987655 has clinical activity in patients with *BRAF* V600-mutated melanoma, *BRAF* wild-type melanoma, and *KRAS*-mutated non-small cell lung cancer (NSCLC), but not in *KRAS*-mutated colorectal cancer. The safety profile of RO4987655 was at the level predicted from the dose escalation Phase I with no new safety signals being identified. Evidence of target modulation and early biological activity were shown amongst all indications independent of mutational status. Further development of RO4987655 based on its toxicity/efficacy profile and early clinical results in this expansion phase may potentially be pursued in *BRAF* wild-type melanoma in combination with other emerging signal transduction inhibitors after immunotherapy failure. Initial observations of clinical activity in *NRAS*-mutated

melanoma need further confirmation. In *KRAS*-mutated NSCLC combinations with taxanes, other cytostatics or PI3K inhibitors and/or mTOR-inhibitors may be rationale approaches for the future.

Purpose: This phase I expansion study assessed safety, pharmacodynamic effects and antitumor activity of RO4987655, a pure MEK inhibitor in selected advanced solid tumor patients.

Experimental Design: We undertook a multicenter phase I two-part study (dose escalation, cohort expansion). Here we present the part 2 expansion that included melanoma, non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) with oral RO4987655 administered continuously at recommended doses of 8.5 mg twice-daily until progressive disease (PD). Sequential tumor sampling investigated multiple markers of pathway activation/tumor effects, including ERK-phosphorylation and Ki-67 expression. BRAF- and KRAS-testing were implemented as selection criteria and broader tumor mutational analysis added.

Results: 95 patients received RO4987655, including 18 *BRAF*-mutant melanoma, 23 *BRAF* wild-type melanoma, 24 *KRAS*-mutant NSCLC, and 30 *KRAS*-mutant CRC. Most frequent adverse events were rash, acneiform dermatitis and gastrointestinal disorders, mostly grade 1/2. Four (24%) of 17 *BRAF*-mutated melanoma had partial response as did four (20%) of 20 *BRAF* wild-type melanoma and two (11%) of 18 *KRAS*-mutant NSCLC. All *KRAS*-mutant CRC developed PD. Paired tumor biopsies demonstrated reduced ERK-phosphorylation among all cohorts but significant differences among cohorts in Ki-67 modulation. 69% showed decrease in fluorodeoxyglucose-uptake between baseline and day 15. Detailed mutational profiling confirmed RAS/RAF screening and identified additional aberrations (*NRAS*/*non-BRAF* melanomas; *PIK3CA*/*KRAS* CRC) without therapeutic implications.

Conclusions Safety profile of RO4987655 was comparable to other MEK inhibitors. Single agent activity was observed in all entities except CRC. Evidence of target

modulation and early biological activity were shown amongst all indications independent of mutational status.

Introduction

The RAS/RAF/MEK/ERK pathway (MAPK pathway) plays a central role in regulating proliferation, differentiation and survival. MAPK-pathway activation can occur through several mechanisms, including mutations in *RAS* or *BRAF* (1, 2). Mutated, oncogenic forms of *RAS* are found in 30% of all human cancers - 45% of colorectal cancer (CRC), 20% of non-small cell lung cancer (NSCLC), 15-20% of melanomas, and 90% of pancreatic cancers (3). *BRAF*-mutations are reported in approximately 50% of cutaneous melanomas, in 40%-70% of papillary thyroid carcinomas, in 5%-20% of CRC, in 10%-20% of cholangiocarcinoma, 1%-5% of NSCLC, and in the vast majority of hairy cell leukemia (4-6).

MEK 1/2 is the only enzyme known to activate ERK 1/2, ERK 1/2 being the only known substrate of MEK; therefore, MEK-inhibition represents an attractive mechanism for blocking MAPK-pathway activation (7). Recently, the MEK inhibitor trametinib has been shown to improve overall survival in *BRAF* V600-mutated metastatic melanoma (8) compared with dacarbazine or paclitaxel chemotherapy and has been approved by the Food and Drug Administration (FDA). Other small-molecule inhibitors of MEK are currently under clinical investigation (9-20). RO4987655 is a potent, highly selective adenosine triphosphate non-competitive oral MEK inhibitor with manageable toxicity profile, favorable pharmacokinetics/pharmacodynamics characteristics, and preliminary antitumor activity in a phase I dose escalation (part 1) study in advanced solid cancers (21). Dose-limiting toxicities (DLTs) were blurred vision (n=1) and elevated creatine phosphokinase (n=3); all of which were reversible without treatment. The maximum tolerated dose (MTD) of RO4987655 was 8.5 mg twice daily. At the MTD, high (mean 75%) and sustained (90% of time >IC_{50}) pERK inhibition was observed in peripheral blood mononuclear cells, and plasma drug concentrations were in the range

predicted to be efficacious in preclinical models. Therefore, a dose regime of 8.5 mg twice daily was recommended for the phase I expansion (part 2) study. The significant incidence of RAS and/or RAF mutations in CRC, in NSCLC, and in melanoma (3-5) associated with favorable preclinical data (12, 14, 18, 20, 21) was the rationale to choose these specific tumor types for the expansion study. The relatively low response rate expected in each tumor type based on previous study data (18, 22) was the rationale for the chosen number of patients treated in each cohort.

The objective of this multicenter part 2 expansion study was to investigate the safety, the pharmacokinetics/pharmacodynamic profile, and single agent antitumor activity of RO4987655 given at defined MTD (21) in diagnostically pre-selected cohorts with RAS or RAF driver mutations, including BRAF V600 mutation carrying melanoma, melanoma without BRAF V600 mutation (including possible other pathway specific aberrations i.e. *NRAS*), *KRAS*-mutated NSCLC, and *KRAS*- and/or *BRAF* V600-mutated CRC. The study was supported by extensive translational/imaging investigations to evaluate biological activity and to identify potential predictive markers for single agent efficacy of RO4987655 in tumors bearing activating mutations in the MAPK pathway.

Materials and Methods

Patients

Eligibility criteria included histologic or cytologic evidence of advanced or metastatic melanoma carrying BRAF V600 mutation, melanoma without BRAF V600 mutation, NSCLC with KRAS mutation, CRC with KRAS- and/or BRAF V600 mutation. Inclusion was restricted to two prior systemic therapies for melanoma and CRC, and

three prior regimens for NSCLC. All prior systemic therapies were permitted. Additional requirements included age ≥18 years, Eastern Cooperative Oncology Group (ECOG) performance status ≤1, life expectancy of ≥12 weeks, measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1(23), adequate bone marrow, renal, hepatic and cardiac function. Mandatory tumor biopsies were obtained at baseline and on day (D)15/cycle (C)1. Patients with history of retinal vein occlusion, glaucoma, central serous retinopathy, corneal erosion, or risk factors for these ocular disorders were excluded. Patients with asymptomatic, corticosteroid free and radiographically stable previously treated brain metastases (≥4 weeks after radiation treatment) were eligible.

Study Design

We performed this multicenter, open-label, phase I study in two parts. In part 1 (dose escalation), the MTD, DLTs, the pharmacokinetics/pharmacodynamic profile, safety, and preliminary antitumor activity of RO4987655 in patients with solid tumors were identified. The results of part 1 were reported after completion (21). Part 2 (first patient in) started eight months after completion of part 1. In part 2 (cohort expansion), we assessed the safety, the pharmacokinetics/pharmacodynamic profile, and single agent antitumor activity of RO4987655 given at defined MTD in patients with RAS/RAF mutations in melanoma, NSCLC and CRC. This part 2 expansion study was conducted at twelve European sites, approved by institutional ethics committees, and conducted in accordance with Declaration of Helsinki/Good Clinical Practice. All patients gave written inform consent. RO4987655 was administered twice daily at 8.5 mg, using a 28D cycle, until progressive disease (PD), intolerable toxicity, or patient withdrawal. For any given patient, a maximum of 1 dose reduction or interruption was allowed after day 28 of cycle 1. Dose reductions were made for grade 3 or other intolerable drug-related toxicity. Re-escalation was permitted for

grade ≥3 skin toxicity and/or isolated creatine phosphokinase elevation which improved to grade ≤2, and for diarrhea or any other toxicity which improved to grade ≤1 within 14 days.

Assessments

Regular assessments, including fundoscopy and of cardiac function, were carried out at baseline and throughout treatment. Disease assessment was performed at baseline and every 8 weeks according to RECIST version 1.1.(23). Adverse events (AEs) were graded according to National Cancer Institute Common Toxicity Criteria (CTC version 3.0).

Pharmacokinetics

Blood samples (4 mL potassium EDTA vacutainers) for pharmacokinetics analysis were collected pre-dose, 0.5 and 10 hours on D1/C1 following drug administration; pre-dose on D8/C1 and pre-dose, 1 and 3 hours postdose on D15/C1. RO4987655 levels were determined by validated liquid chromatography/tandem mass spectrometry (LC/MS/MS)(24). Pharmacokinetic parameters were calculated via standard noncompartmental methods using WinNonlin V6.1(Pharsight Corporation).

Pharmacodynamics

Tumor biopsies. RO4987655 effects on cellular proliferation (Ki67- labeling) and target/pathway inhibition (pERK-expression) were investigated by immunohistochemistry in paired tumor biopsies (at baseline and D15/C1). A ≥20% change in biomarkers between baseline and D15/C1 was considered relevant.

Immunohistochemistry. Tumor biopsies were formalin fixed and paraffin embedded following standard procedures. Immunohistochemistry for Ki67 and pERK was conducted using ultraView detection kit (Ventana Medical Systems Inc.) and iView detection kit (Ventana) on the Ventana Benchmark XT platform according to the manufacturer's instructions (21).

Tumor-DNA Mutation Analysis. Mutational analysis for patient selection was performed centrally using formalin-fixed archival tissue samples which were collected prior to the start with R04987655. Biopsies were microscopically assessed to ensure ≥50% tumor content and manually microdissected. Real-time PCR with fluorescence-labeled, sequence-specific probes was used to distinguish wild-type (WT) BRAF (V600) sequence (GTG) from mutant sequence (GAG). KRAS-mutations were identified using an investigational assay based on PCR/melting point analysis, with fluorescence-labeled, sequence-specific probes to distinguish WT-sequence from mutation bearing sequences in exon 2 (codons 12/13) and exon 3 (codon 61). All assays were performed on Cobas 4800 system (Roche Molecular Systems, Inc.) according to manufacturer's instructions. Mutational analysis for 19 oncogenes and 225 somatic mutations (Supplementary Figure 1) was also conducted in available DNA from fresh tumor biopsies and/or archival tumor samples at baseline using the Sequenom OncoCarta panel v1.0 (25). All 19 genes/ selected 225 somatic mutations were tested in all samples and called as "non-mutated" if not otherwise reported.

Fluorodeoxyglucose Positron Emission Tomography (FDG-PET). Metabolic activity was investigated by FDG-PET at baseline, D15/C1 and D1/C3. Baseline and follow-up PET-scans were conducted on the same scanner under identical conditions. Low-dose CT-scans for all PET-scans served for attenuation correction. Independent analysis of PET-images was conducted centrally based on EORTC-guidelines(26). Lesions (≤ 5) with highest FDG-uptake were selected at baseline for quantitative analysis using 10 mm circular regions of interest centered on maximum FDG-uptake to define standardized uptake value (SUV). Changes in SUV between baseline and D15/C1 were calculated for each patient. Patients with recent history of diabetes were excluded from FDG-PET.

Statistical Methods

This report includes results based on the data cutoff of September 21, 2012. The effective dose and antitumor activity were evaluated based on clinical benefit rate (CBR, defined as complete response (CR), partial response (PR) and stable disease (SD)), objective response rate (ORR, defined as CR/PR), and duration of response. Duration of response lasted from date of PR/CR to date of PD or date of death. Best overall response of SD was defined as SD lasting ≥16 weeks for melanoma/CRC and ≥8 weeks for NSCLC. Duration on treatment was defined as the time from first dose to the earlier date of last dose or data cutoff (September 21, 2012), whichever came first. Pharmacokinetics, pharmacodynamics, safety, and tumor response were analyzed by descriptive statistics. Predictive/correlative analyses were performed comparing antitumor activity and biomarkers assessed by logistic regression and ANOVA. All analyses were stratified by tumor type/mutational status. Two sided p-values were evaluated and a p-value of <0.05 was considered statistically significant.

Results

Patients

Between March 2011 to September 2012, 96 patients were enrolled, 95 of whom received RO4987655 (Table 1). All had received previous anti-cancer treatment, including chemotherapy, immunotherapy, targeted agents, surgery, and radiotherapy (Table 1). None had previously received MEK inhibitor but two patients with *BRAF*-mutant melanoma and one patient with *BRAF* wild-type melanoma had undergone previous BRAF inhibitor treatment.

Toxicities/dose modifications/interruptions

Ninety three patients (98%) experienced one or more treatment-related AEs. Table 2 shows the most common AEs (≥10% of patients). Most common treatment-related AEs were skin-related - rash and acneiform dermatitis, gastrointestinal disorders -

diarrhea, nausea and vomiting, general symptomatic disorders - peripheral edema and asthenia, and eye disorders - in particular serous retinal detachment (SRD) and blurred vision (Table 2). Forty three patients (45%) developed SRD. Grade 1 chorioretinopathy and retinal vein occlusion were reported in 2 patients (2%) each. The majority of AEs were mild or moderate in severity and their incidence was comparable across all four cohorts. Sixty one patients (64%) of 95 had treatment-related grade 3 AEs, the majority of which were asymptomatic blood CPK increase (n=16; 17%), rash (n=15; 16%), diarrhea (n=8; 8%), folliculitis (n=7; 7%), and SRD (n=6; 6%). Eight (8%) had treatment-related grade 4 AEs (asymptomatic blood CPK-increase in six (6%), SRD in one (1%), and superinfected dermatitis in one (1%)). Overall 57 serious adverse events (SAEs) were reported in 46 patients (48%). Treatment-related SAEs were noted in 24 (25%), including pulmonary embolism, mucosal inflammation, SRD, and hypokalemia in two each. All other treatment-related SAEs were reported only one each. Adverse events leading to withdrawal of RO4987655 were noted in 12 patients (13%), including intolerable rash (n=3; 3%), observed grade 2 decrease in left ventricular ejection fraction (n=3; 3%) and dyspnea (n=3; 3%). Sixty four patients (67%) of 95 had dose modifications and/or temporary drug interruptions due to AEs, most frequently asymptomatic blood CPK-increase (n=20; 21%), rash (n=18; 19%), SRD (n=14; 15%), and diarrhea (n=8; 8%). There were no treatment-related deaths.

Pharmacokinetics

Pharmacokinetics results were in-line with those reported earlier (21). Plasma concentrations of RO4987655 increased rapidly following oral administration. The apparent systemic clearance was approximately 3.7 L/hour. Plasma exposure appears to be dose-linear, with a coefficient of variation of approximately 50%. Mean

terminal half-life was approximately 12 hours. The effective half-life of RO4987655 was about nine hours

Pharmacodynamics

In total 81 pre-dose and 62 post-dose tumor samples were available for pharmacodynamic analysis. However, only 45 paired biopsies of 95 treated patients (47%) were assessable for pERK expression and 38 paired biopsies (40%) assessable for Ki67. Thus, tumor biomarker results could only be obtained from about 50% of the treated patients. A significant down-regulation of pERK was observed across all four cohorts between baseline and D15/C1 (*BRAF*-mutant melanoma $p<0.007$; *BRAF*-wt melanoma $p<0.002$; NSCLC $p<0.009$; CRC $p<0.0002$). However, a significant reduction of Ki67-expression between baseline and D15/C1 was only seen in *BRAF*-mutant ($p<0.02$) and *BRAF* wild-type melanoma ($p<0.02$) (Figure 1, Supplementary Figure 2).

FDG-PET analyses

FDG-PET analysis was conducted in 74 patients. A decrease in FDG-uptake between baseline and D15/C1 was observed in 51 patients (69%) but in only 15 of 57 patients (26%) studied at D1/C3. The largest FDG-uptake-reduction was observed in melanoma (Figure 1, Supplementary Figure 2). Larger FDG-response was indicative of higher RECIST response rate. Patients without FDG-PET-response on D15/C1 were also not responding per RECIST at a later time point (high negative predictor value). D15/C1 FDG-PET-response was not predictive of later RECIST response with only 10 of 51 patients (20%) showing a decrease in FDG-uptake D15/C1 achieving a RECIST response (low positive predictor value).

DNA Mutational Analysis

Exploratory mutational analyses were conducted for 78 tumor samples: 17 *BRAF* V600-mutant melanoma, 18 *BRAF* wild-type melanoma, 18 *KRAS*-mutant NSCLC

and 25 *KRAS*-mutant CRC. Sixty-eight samples revealed mutations (Figure 2, Table 3): eight NRAS mutations were additionally identified among the 18 *BRAF* wild-type melanoma patients; thirteen BRAF V600 single mutations were confirmed plus four additional concomitant mutations (located on the CDK-, EGFR-, MET- and RET-genes, respectively) were identified in 17 *BRAF* V600-mutant melanomas; fourteen KRAS single mutations plus seven KRAS/PIK3CA double mutations were detected in 21 out of 25 *KRAS*-mutant CRCs (4 samples did not provide enough material for analysis, hence PIK3CA status is unknown); 17 KRAS mutations and one double KRAS/AKT1 mutation were found in 18 *KRAS*-mutant NSCLCs. Figure 2 and Table 3 show the available mutational status of tumors and their response rate.

Antitumor Activity

In total 80 of 95 patients were evaluable for efficacy assessment (Table 3). PR was achieved in four (23.6%) of 17 patients with *BRAF* V600-mutant melanoma (Figure 2A, Table 3), including one receiving study treatment ≥52 weeks (Figure 3). SD for ≥16 weeks was noted in a five patients (29 %). The two patients who were treated previously with vemurafenib experienced PD (Table 3). In 20 patients with melanomas without *BRAF* V600 mutation, four (20%) PRs and four (20%) SD were reported (Figure 2B, Table 3). The patient with *NRAS*-mutant melanoma who experienced PR received study treatment for 323 days. In 18 cases of *KRAS*-mutant NSCLC two PRs were confirmed, including one on treatment for 56 weeks, as well as 8 (44%) with SD ≥8 weeks (Figure 2C, Table 3). All 25 patients with *KRAS*-mutant CRC had PD (Figure 2D, Table 3). The median duration of treatment across all subgroups was 63 days (range 5-394) with the longest median duration of treatment being in *BRAF*-mutant melanomas at 113 days (range 18-366) (Figure 3, Table 3)

Discussion

Our findings show that RO4987655 has clinical activity in patients with *BRAF* V600-mutated melanoma, *BRAF* wild-type melanoma, and *KRAS*-mutated NSCLC, but not in *KRAS*-mutated CRC. The safety profile of RO4987655 was at the level predicted from the Phase I/part 1 selection of the MTD with no new safety signals being identified. Evidence of target modulation and early biological activity were shown amongst all indications independent of tumor mutation status

Similar to other MEK inhibitors (12, 18, 20) rash and diarrhea were the most frequent treatment-related adverse events. The most common ocular toxicity was SRD occurring in 45% of all patients. The incidence of SRD was higher compared to other MEK inhibitors (17, 18) which may reflect the high rate and systematic monitoring of eyes disorders in the study. The mechanism of MEK-related eye disorders remains unknown and should be monitored closely in trials with similar compounds.

Evidence of pharmacodynamic drug effect was observed with a metabolic response in all four cohorts, with markedly larger effects in those in which objective tumor shrinkage was observed. Our study confirms the high negative predictive value of FDG for MEK inhibition and potential for FDG-PET to predict early non-responders (27). We also noted a significant decrease of pERK-phosphorylation in all cohorts, but an effect on proliferation (as measured by Ki67) was only observed in the two melanoma cohorts. These results may indicate an attenuated effect on the tumor by RO4987655 in *KRAS*- mutant NSCLC and particularly in CRC. However, it should be taken into consideration that the tumor biomarker results (assessed by immunohistochemistry) were only obtained from about 50% of the treated patients due to limitation in the amount of tumor materials. The high FDG response on D15/C1 coupled with limited effects on cellular proliferation suggests that change in

FDG uptake might not be related reduction in the number of viable tumor cells but instead reflects an effect on glucose uptake and utilization. This effect is rapid, reversible and does not appear sufficiently sustained to induce apoptosis or necrosis and generate a response at later time points.

Current treatment progress in advanced melanoma has been focusing on relatively common *BRAF* V600-mutations (>50% melanomas) (4). Recently, the BRAF inhibitors vemurafenib and dabrafenib, the MEK inhibitor trametinib, and the combination of the MEK inhibitor, trametinib and the BRAF inhibitor, dabrafenib have been approved for the treatment of *BRAF* V600-mutated metastatic melanoma based on Phase II/III clinical trials (8, 28-30). Besides BRAF-inhibition, downstream targeting of MAPK pathway has evolved as a further interesting target. Recently, the MEK inhibitor MEK162 has shown activity in melanoma harboring NRAS- or V600 BRAF-mutation (17). A further MEK inhibitor currently under investigation in advanced melanoma and in *GNAQ/GNA11*-mutant uveal melanoma is selumetinib (19, 31, 32). Our study demonstrated clinical efficacy with the MEK inhibitor RO4987655 both in *BRAF* V600-mutated melanoma but also non *BRAF* V600-mutated melanoma, including *NRAS*-mutated melanoma. However, based on the well-established role of the cytotoxic T-lymphocte antigen 4 (CTLA-4) inhibitor ipilimumab (33, 34) and the emerging data with the new immune checkpoint inhibitors, i.e. programmed death 1 (PD-1) (35-37)and programmed death ligand 1 (PD-L1) inhibitors (38, 39), as well as the combination of both (40), the role of RO4987655 as single agent in patients with *BRAF* wild-type melanoma is limited. Further, the anti-PD-1 antibody, nivolumab, is being tested in a pivotal registration phase III trial as monotherapy versus dacarbazine in patients with *BRAF* wild-type metastatic melanoma (NCT01721772). If successful, this trial will establish an attractive future option for patients with *BRAF* wild-type melanoma. However, a small

window of opportunity for combinations with MEK inhibitors exists for *BRAF* wild-type patients progressing following initial immunotherapy. Since a recent double blind randomized phase II trial of docetaxel with or without the MEK inhibitor selumetinib in *BRAF* wild-type melanoma showed no significant improvement in progression free survival compared with docetaxel alone (41), chemotherapy combinations may not be the best choice for this population. A possible strategy in *BRAF* wild-type melanoma after immunotherapy failure could be a combination of a MEK inhibitor with other new emerging signal transduction inhibitors. Moreover, in *BRAF* V600-mutant melanoma, the combination of the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib is superior to single agent treatment (30).

MEK inhibitors and BRAF inhibitors are under investigation in pretreated patients with advanced NSCLC. Single agent selumetinib showed limited activity in a randomized Phase II versus single agent pemetrexed in unselected second-line patients with NSCLC (42). Data from NSCLC cell lines strongly suggest that those with RAS mutations are sensitive to selumetinib (43). Therefore, a signal finding randomized Phase II was performed comparing the combination of docetaxel and selumetinib in *KRAS*-mutated NSCLC with docetaxel alone. The combination gave a better response rate (40% vs. 0%) and significantly improved progression free survival (44). Toxicity of the combination has led to an ongoing randomized Phase II trial (NCT01750281) investigating different docetaxel doses in combination with MEK inhibitor versus docetaxel monotherapy to better understand the toxicity/efficacy ratio of combinations of MEK inhibition and chemotherapy. The promising CBR ≥ 8 weeks of 56% (10/18; 2 PR/8 SD) with the MEK inhibitor RO4987655 as single agent in 18 patients with *KRAS*-mutated NSCLCs in third-line and significant metabolic responses demonstrated with FDG-PET/CT in this cohort underline that further investigation of MEK inhibitors preferably in combination with conventional cytotoxics

and/or targeted agents in *KRAS*-mutated NSCLC may be justified. However, given the fact that patients with wild-type NSCLC were excluded from this expansion study, it is not possible to determine whether activity of RO4987655 in NSCLC is related to KRAS mutation status. This is particularly important, since in melanoma, activity was also seen in *BRAF* wild-type patients. Furthermore, a phase I/Ib study combining the oral MEK inhibitor trametinib with docetaxel or pemetrexed in *KRAS*-mutant and wild-type NSCLC demonstrated tolerability and clinical activity in both settings (NCT01192165) (45, 46). 1-5% of NSCLCs harbor BRAF V600 mutations (4, 5). In these patient groups BRAF inhibitors are currently being investigated (NCT01336634) (47). Combinations of MEK inhibitors with BRAF inhibitors may also be of interest.

Despite metabolic responses on FDG-PET/CT we saw no clinical activity in *KRAS*-mutant CRC. The effect on Ki67-expression in paired biopsies was also not significant, including a few cases with up-regulation of Ki67 in matched on-treatment samples. Coexistence of KRAS- and PIK3CA mutations has been demonstrated in CRC-tumors (48). Thus single agent administration of MEK inhibitors in CRC may not be advisable based on primary resistance mechanisms, but combinations with other signal transduction inhibitors may yet prove effective. In our study, we observed that the majority of tumors bearing double activating PIK3CA/KRAS mutations cluster among the less responsive patients. Despite insufficient sample size to derive robust conclusions, this may identify a particular aggressive tumor phenotype displaying double mutations and this would indicate combined treatment with PI3K inhibitors. In addition, preclinical results also predict primary resistance of CRC-cell lines to MEK inhibitors and confirm that there is a strong biological rationale to combine these drugs with PI3K inhibitors or mTOR inhibitors (49, 50).

In conclusion, further development of MEK inhibitor RO4987655 based on its toxicity/efficacy profile and early clinical results in this expansion phase I may potentially be pursued in *BRAF* wild-type melanoma in combination with other emerging signal transduction inhibitors after immunotherapy failure. .Initial observations of clinical activity in *NRAS*-mutated melanoma need further confirmation. In *KRAS*-mutated NSCLC combinations with taxanes, other cytostatics or PI3K inhibitors and/or mTOR inhibitors may be rationale approaches. In CRC MEK inhibitor monotherapy is not effective and combinations with PI3K- and/or mTOR inhibitors may potentially be of interest.

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TABLES

Table 1: Patients Characteristics

Characteristics	Melanoma BRAF V600 mutated (n=18)		Melanoma not mutated for BRAF V600 (n=23)		NSCLC KRAS mutated (n=24)		CRC KRAS mutated (n=30)	
	No.	%	No.	%	No.	%	No.	%
Age, years (Median[range])	47 (20-47)		55 (29-80)		55 (44-69)		57 (36-86)	
Weight (kg) (Median[range])	71 (52-94)		67 (51-98)		67 (45-102)		70.5 (43-166)	
Sex (Male/Female)	7/11	39/61	10/13	43/57	5/19	21/79	14/16	47/53
Race (White/Black)	18/0	100/0	23/0	100/0	24/0	100/0	29/1	97/3
ECOG baseline								
0/1/2/NK	10/8/0/0	56/44/0/0	16/7/0/0	70/30/0/0	7/14/1/2	30/58/4/8	13/16/0/1	43/54/0/3
No. of metastatic sites								
0/1/2/≥3	0/2/2/14	0/11/11/78	0/2/6/15	0/9/26/65	1/1/5/17	4/4/21/71	0/2/13/15	0/7/43/50
Previous brain metastases	3	17	0	0	6	25	0	0
Prior anticancer treatments								
Surgery	17	94	18	78	8	33	24	80
Radiation	8	44	11	48	11	46	5	17
Prior systemic therapies[+]								
0/1/2/≥3	6/4/4/4	33/22/22/22	5/7/10/1	22/30/43/4	0/2/12/10	0/8/50/42	0/1/24/5	0/3/80/17
Previous BRAF inhibitior	2	11	1	4	0	0	0	0
Previous anti-CTLA- 4 AB[++]	5	28	3	13	0	0	0	0

Abbreviations: NSCLC, non-small cell lung cancer; CRC, colorectal cancer; ECOG, Eastern Cooperative Oncology Group; No, number; NK, not known; anti-CTLA-4 AB, anticytotoxic T-lymphocyte-associated protein 4 antibody

[+] Including prior chemotherapy and/or immunotherapy and/or targeted agents; [++]Defined as previous therapy with Ipilimumab or tremelimumab

Table 2: Severity of drug-related adverse events reported in more than 10% of the study patients

	All adverse events	Related adverse events, n(%)	
	n (%)	Grade 1/2	Grade 3/4
Skin-related toxic effects Rash, folliculitis, dry skin	90 (95)	79 (83)	25 (26)
Gastrointesinal disorders Diarrhea, nausea, vomiting	84 (88)	77 (81)	13 (14)
General disorders Oedema, asthenia, fatigue, mucosal inflammation	80 (84)	59 (62)	12 (13)
Eye disorders Serous retinal detachement, blurred vision	67 (71)	58 (61)	9 (10)
Laboratory abnormalities CPK increased	45 (47)	25 (26)	25 (26)
Respiratory, thoracic and mediastinal disorders Cough, dyspnoe, pleural effusion, pulmonary embolism	45 (47)	13 (14)	5 (5)
Infections Oral candidiasis, subcutaneous abscess, cellulitis	37 (40)	13 (14)	3 (3)
Nervous system disorders Headache, dysgeusia	32 (34)	16 (17)	2 (2)
Metabolism and nutrition disorders Decreased appetite, hypocalcaemia	26 (27)	14 (15)	2 (2)

Abbreviations: CPK, creatine phosphokinase

Table 3: Clinical activity

	Patients	Overall Response						
	(n)	PR (%)	PR unconfirmed	PR confirmed	SD[+] (%)	not assesable for SD	PD (%)	CBR[++] (%)
Melanoma BRAF V600 mutated^	17	4 (24)	1	3	5 (29)	0	8 (47)	9 (53)
Previous BRAF inhibitor	2	0			0		2 (100)	
Melanoma not mutated for BRAF V600	20	4 (20)	1	3	4 (20)	1	11 (55)	8 (40)
NRAS	8	1(13)	0	1	2 (25)	0	5 (62)	3 (38)
non-NRAS	10	2 (20)	1	1	2 (20)	1	5 (50)	4 (40)
NRAS unknown	2	1 (50)	0	1	0	0	1 (50)	1 (50)
NSCLC KRAS mutated^^	18	2 (11)	0	2	8 (44)	0	8 (44)	10 (56)
CRC KRAS mutated	25	0	0	0	0	1	24 (100)	0
KRAS^^^	14	0	0	0	0	0	14 (100)	0
KRAS/PIK3CA	7	0	0	0	0	0	7 (100)	0
KRAS mutated/PIK3CA status unknown	4	0	0	0	0	1	3 (100)	0

Abbreviations: NA, not applicable; PR, partial response; SD, stable disease; PD, progressive disease; CBR, clinical benefit rate; NSCLC, non-small cell lung cancer; CRC, colorectal cancer

[+] best overall response of SD was defined as SD lasting ≥16 weeks for melanoma/CRC and ≥8 weeks for NSCLC
[++] defined as complete response, partial response and stable disease
^ Inclusive BRAF V600/CDK, BRAF V600/RET, BRAF V600/EGFR, BRAF V600/MET
^^ Inclusive KRAS/AKT1
^^^ Inclusive KRAS/CDK and KRAS/MET

FIGURE LEGENDS

Figure 1

Biological activity across the four cohorts. Evidence of early biological effect, as measured by FDG-PET was shown for all four cohorts with markedly lower effect in colorectal cancer than in other tumor types. Evidence of pathway inhibition effects (%pERK) was observed across all cohorts with strongest effect in patients with *BRAF* V600-mutant melanoma. Early evidence of pharmacodynamic effects and blockade of cellular proliferation (%Ki67 decrease) was only observed in patients with *BRAF* V600-mutant melanoma.

Figure 2

Tumor percent change from baseline and exploratory genotyping in the four cohorts

(A) *BRAF* V600-mutant Melanoma

(B) Non *BRAF* V600-mutant melanoma

(C) *KRAS*-mutant non-small cell lung cancer

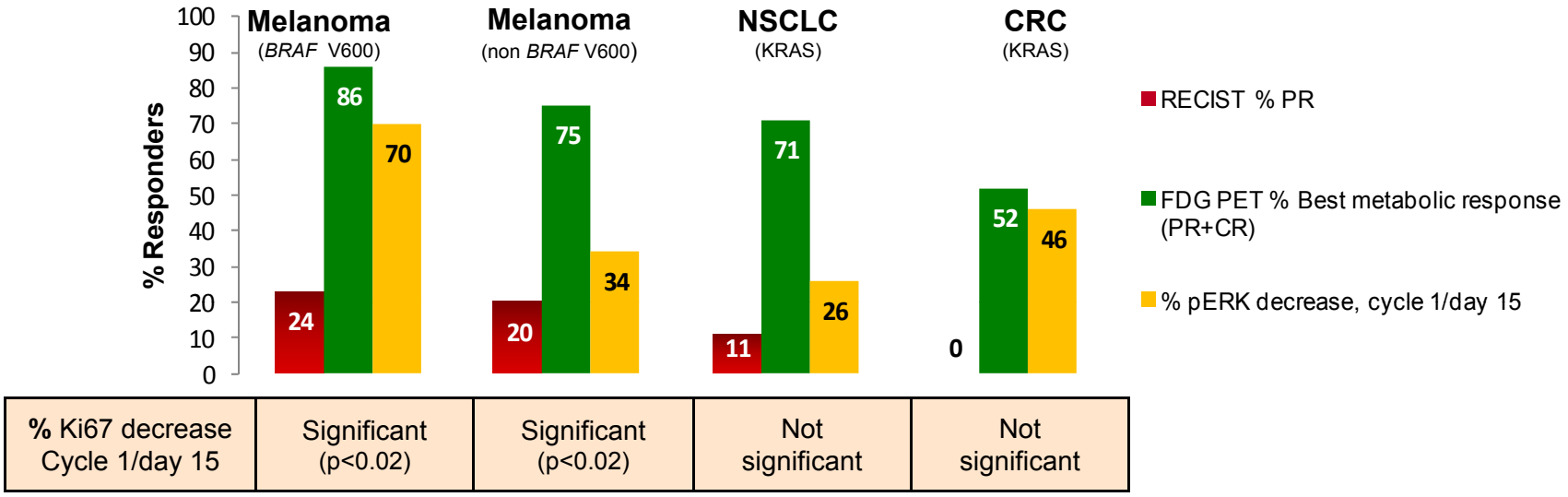
(D) *KRAS*-mutant colorectal cancer

Figure 3

Duration on treatment in the four cohorts.

Figures

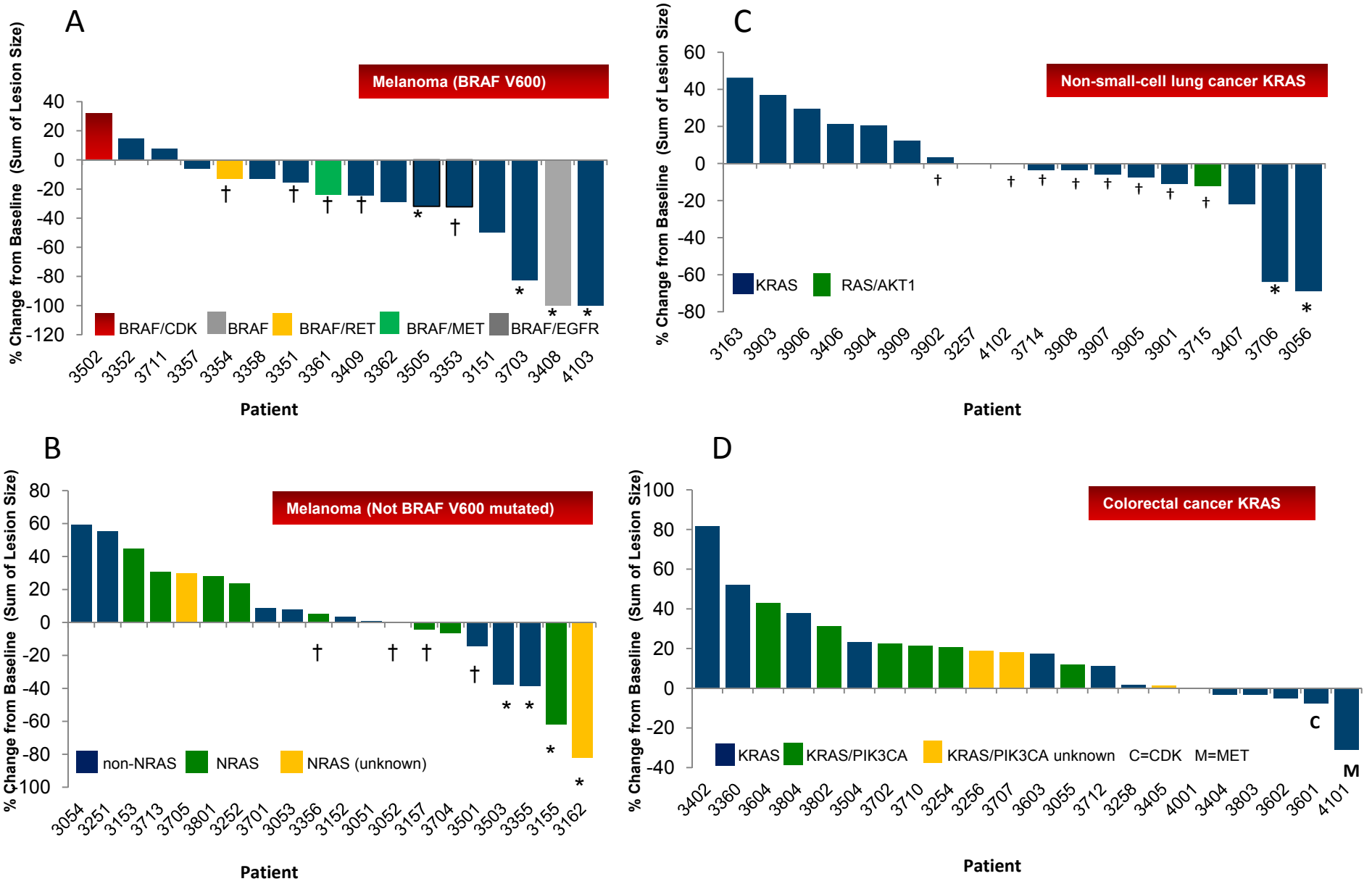
Figure 1



% Ki67 decrease Cycle 1/day 15	Significant (p<0.02)	Significant (p<0.02)	Not significant	Not significant

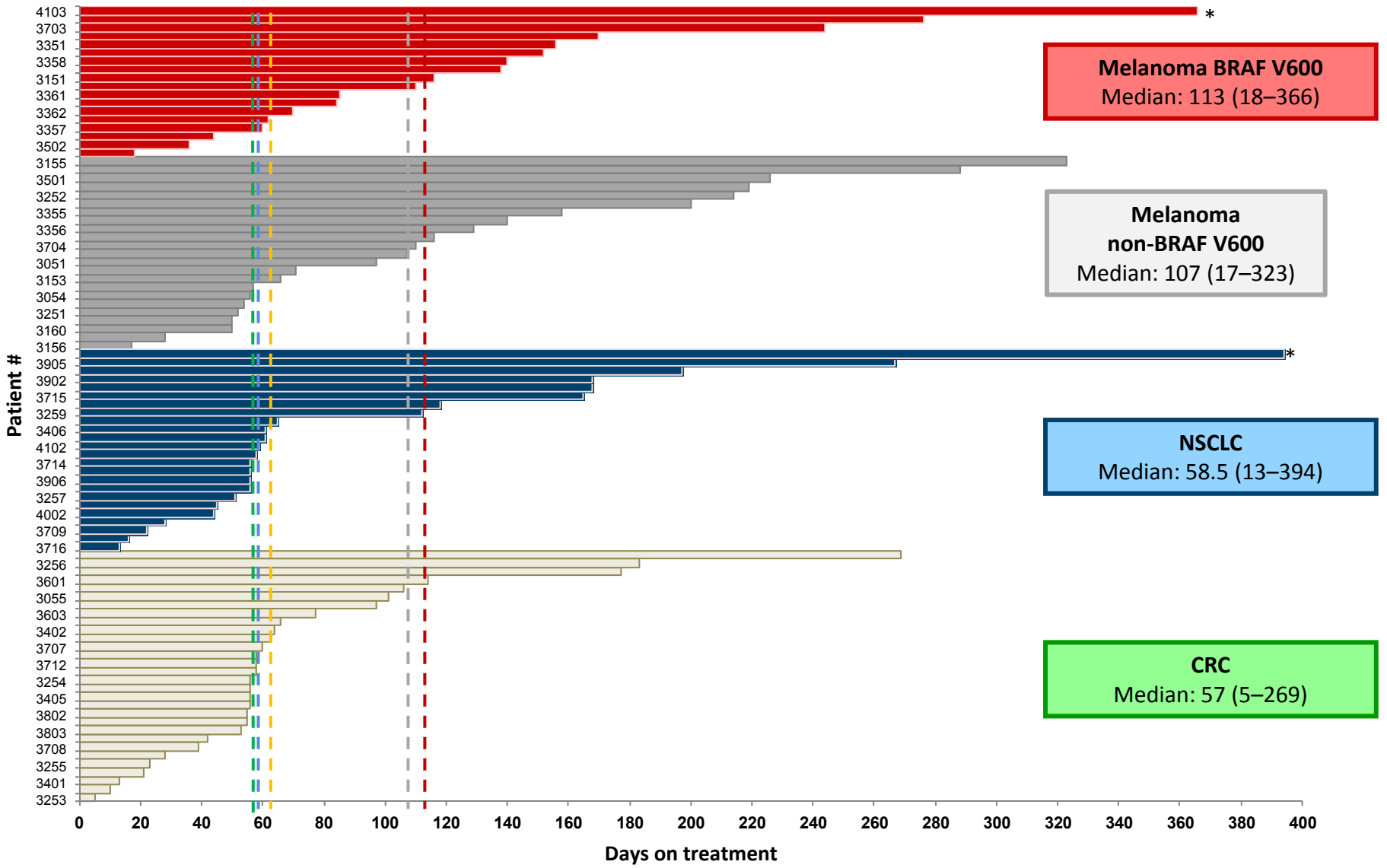
CRC, colorectal cancer; **FDG PET**, fluorodeoxyglucose positron emission tomography; **NSCLC**, non-small cell lung cancer; **pERK**, phosphorylated extracellular-related kinase; **RECIST**, Response Evaluation Criteria In Solid Tumors

Figure 2



*Partial Responders; †Stable Disease

Figure 3



*2 pts ongoing:
- NSCLC KRAS mutated (56 weeks on treatment, actual dose 5 mg BID)
- Melanoma BRAF V600 mutated (52 weeks on treatment, 8.5 mg BID)

AACR American Association for Cancer Research

Clinical Cancer Research

Phase I expansion and pharmacodynamic study of the oral MEK inhibitor RO4987655 (CH4987655) in selected advanced cancer patients with RAS-RAF mutations

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