# EARLY CLINICAL DEVELOPMENT OF TARGETED ANTICANCER AGENTS

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# EARLY CLINICAL DEVELOPMENT OF TARGETED ANTICANCER AGENTS

Klinische ontwikkeling van doelgerichte antikanker therapieën

(met een samenvatting in het Nederlands)

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# PREFACE

Cancer is the disease for which most drugs are being developed at the moment[1]. In 2016, about 40% of the newly approved drugs in Europe were for the treatment of cancer[2,3].

Initially, most anticancer drugs belonged to the group of cytotoxic anticancer drugs which exert their anticancer effect by interfering with cell replication and survival, mainly in rapidly replicating cells. Over the last years, the focus was shifted to development of the so-called targeted agents. Targeted agents are designed to target specific proteins that are overexpressed by or mutated in cancer cells or proteins that induce growth of cancer cells. Promising anticancer effects of targeted therapies have been reported. Unfortunately, tolerability of these agents and treatment resistance remain major hurdles. In 2013, a novel strategy garnered attention after the high impact journal 'Science' rewarded immunotherapy for cancer as the breakthrough of the year[4]. Immunotherapies are designed to activate immune-responses towards cancer cells and hereby enable destruction of cancer cells by the immune system. Although immunotherapies are often defined as a separate group, they also belong to the group of targeted anticancer agents.

With the use of targeted therapies, the concept of 'personalized medicine' became increasingly relevant. Targeted therapies enable selection of patients who are most likely to benefit from treatment. The applied selection criteria are usually defined as biomarkers, which are included in all studies described in this thesis. Although this concept of personalized medicine based on biomarkers sounds promising, this approach can only be successful when some conditions are met. First, the right biomarkers should be used, to allow adequate selection of patients. Secondly, the drug should reach its target, mostly a specific protein in or around the tumor cells, to induce an anti-tumor effect. The third important factor for every drug is the balance between efficacy and toxicity. Especially for anticancer drugs, the search for the optimal efficacy and tolerability can be complicated. Fourthly, efficacy can be enhanced by combining two or more agents. Combinations can induce a synergistic antitumor effect but can also delay or avoid resistance, which often occurs fast in single agent therapies.

This thesis focuses on the early stage of clinical development of novel anticancer agents with a focus on phase I trials. Phase I trials often cover the first-in human application of a novel agent or a novel combination, in which the aim is to evaluate the safety of the drug or combination and to determine the optimal dose and regimen. If feasible, phase I trials are followed by phase II, III and IV trials, which focus more on the efficacy and safety in large populations and compare the novel drug to existing therapies. We describe the results of phase I trials with targeted anticancer agents and present recommendations for the design of future phase I trials, focusing on the challenges that have to be faced now and in the future in early clinical development.

**Chapter 1** focuses on general aspects of early development of anticancer agents. In **Chapter 1.1**, the design of phase I clinical trials with targeted anticancer agents is reviewed. We discuss how the design of phase I trials relates to their performance in terms of trial duration and the number of patients needed. **Chapter 1.2** focuses on immunotherapies. One of the challenges in the development of immunotherapeutic drugs is their immunogenicity. After administration, immune responses can occur in which antibodies are formed that are directed towards the drug (anti-drug antibodies). We discuss the incidence, consequences and strategies to deal with anti-drug antibodies in future clinical trials.

In **chapter 2,** the results of combining targeted agents to overcome treatment resistance is described. Chapter **2.1, 2.2** and **2.3** are based on results of three phase I studies with MEK and pan-HER inhibitors. In **chapter 2.4**, data from these three studies are merged in order to explore the relationship between exposure to MEK and HER inhibitors and toxicity. Based on a pharmacokinetic-toxicodynamic model, recommendations for rational dosing strategies for these combinations are provided.

In **chapter 3,** three phase I trials with immunotherapies are described. **Chapter 3.1** provides results on the drug disposition of a novel immunocytokine, consisting of a carcinoembryonic antigen (CEA) antibody and an interleukin-2 variant, in a first-in-human setting. **Chapter 3.2** and **3.3** focus on the efficacy and safety of the anti-programmed death (PD) 1 antibody pembrolizumab in a variety of solid tumors with confirmed PD-ligand (L) 1 expression.

**Chapter 4** describes the utility of *BRAF* status as a biomarker for response to treatment with anti-EGFR monoclonal antibodies. Several studies indicate that mutations in the *BRAF* gene induce resistance to treatment with these agents. By upfront screening on genetic alterations in *BRAF*, we may be able to select patients for whom maximum treatment benefit is expected. In this chapter, we discuss the impact of these mutations on treatment response.

Finally, a reflection on the combined results of the research described in this thesis is presented in **chapter 5**. Herein, the future perspectives of previously described anticancer agents and the future challenges in early clinical development are discussed.

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“Per aspera ad astra”

# Chapter 1

# Strategies for development of novel anticancer therapies

## Chapter 1.1

**The performance of model-based versus rule-based phase I clinical trials in oncology**
A quantitative comparison of the performance of model-based versus rule-based phase I trials with molecularly targeted anticancer drugs over the last two years

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**ABSTRACT**

Phase I studies with anticancer drugs are used to evaluate safety and tolerability and to choose a recommended phase II dose (RP2D). Traditionally, phase I trial designs are rule-based, but for several years there is a trend towards model-based designs. Simulations have shown that model-based designs perform better, faster and are safer to establish the RP2D than rule-based designs. However, the superiority of model-based designs has never been confirmed based on true trial performance in practice. To aid evidence-based decisions for designing phase I trials, we compared publications of model-based and rule-based phase I trials in oncology. We reviewed 172 trials that have been published in the last two years and assessed the following operating characteristics: efficiency (trial duration, population size, dose-levels), patient safety (dose-limiting toxicities (DLTs)) and treatment optimality (percentage of patients treated below and at or above the recommended phase 2 dose). Our results showed a non-significant but clinically relevant difference in trial duration. Model-based trials needed ten months less than rule-based trials (26 months versus 36 months; $p = 0.25$). Additionally, fewer patients were treated at dose-levels below the RP2D (31% versus 40%; $p = 0.73$) while safety was preserved (13% DLTs versus 14% DLTs). In this review, we provide evidence to encourage the use of model-based designs for future phase I studies, based on a median of ten months of time gain, acceptable toxicity rates and minimization of suboptimal treatment.

## INTRODUCTION

Phase I trials investigate the safety, tolerability, and preliminary efficacy of novel agents or combinations. In oncology, the primary goal of these trials is to determine the recommended dose in patients, known as the recommended phase II dose (RP2D), for use in a follow-up trial. It is commonly acknowledged that phase I trials should identify an accurate RP2D while minimizing sub-therapeutic treatment or toxic treatment. These operating characteristics depend on the trial design (i.e. escalation method), so careful consideration of the design is crucial.

Traditionally, dose-escalation has been conducted according to the 3+3 principle and its variants. In these rule-based designs, dose-levels are chosen according to a pre-specified rule or algorithm[1, 2]. Although the use of rule-based designs is still prevailing, model-based designs such as the continual reassessment method (CRM) gain popularity in clinical practice[1, 3–5]. In these designs, dose-levels are determined by estimating a model for the dose–toxicity relationship. Based on results from simulations, model-based designs are considered to have several advantages over classical rule-based designs, such as shorter trial duration[1], minimal suboptimal treatment[5] and a more accurate estimation of the RP2D[2, 5, 6]. Both the European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA) have recommended the use of model-based designs in order to improve phase I trial performance[7, 8]. However, the practical performance of rule-based and model-based trials has never been compared, hindering evidence-based decision making on trial design.

Our objective is to fill this gap in literature by providing a quantitative comparison of the performance of rule-based versus model-based oncological phase I trials based on a comprehensive systematic review of literature. We provide an overview of the theoretical and practical performance of rule-based and model-based phase I trials, which can be used for decision-making and future research. The main question to be addressed is whether model-based designs are indeed superior to classical rule-based designs.

## THEORETICAL

### Rule-based designs

Rule-based designs have been considered a safe and easy-to-implement approach to determine the RP2D[1]. The most commonly used rule-based designs are the 3+3 design and its variations including the 6+6 design, accelerated-titration and pharmacologically-guided-dose-escalation (PGDE)[1, 2]. The characteristic of rule-based designs is that dose-escalation is guided by predefined rules based on the actual occurrence of dose-limiting toxicities (DLTs) among patients at the last dose-level. In the 3+3 design for example, three patients are included in each cohort, with escalation to the next higher dose-level if no DLT occurs and expansion to six patients if one of the first three subjects develops a DLT. If upon expansion no additional DLTs are reported, the dose will be escalated and the same rule applies. Escalation stops if two or more out of six patients (or out of three) experience a DLT. The previous lower dose-level is then expanded to six patients. The dose at which at most one out of six patients experiences a DLT is considered the maximum tolerated dose (MTD) or recommended phase II dose (RP2D). The 3+3 design has been generally accepted as a safe dose-escalation approach. The incidence of DLTs among all included patients (toxicity rate) in rule-based trials should be at most 33%. At any dose-level, less than two out of six patients should experience a DLT. The same holds true for the final RP2D in rule-based trials[1].

Previously, it has been reported that rule-based trials need many dose-escalation steps to find the RP2D. This may result in excessive treatment at low (suboptimal) doses[1,9], large population sizes[10] and long trial duration[1,5]. In addition, the established RP2D may be too low, as shown by Zhou *et al.* who reported that the toxicity rate of the RP2D identified by rule-based trials may vary between 10 and 29%[3,11].

### Model –based designs

The first well known model-based design was introduced as the continual reassessment method (CRM)[12]. Variations on the CRM include the escalation with overdose control (EWOC) design and the time-to-event CRM (TITE-CRM)[1]. For a detailed description of these designs we refer to reviews by Jaki *et al.*[2] or Le Tourneau *et al.*[4].

In model-based designs, dose-escalation is guided by a model describing the dose-toxicity relationship. By repeatedly incorporating toxicity data from all explored doses including data from previous trials if they are available, an estimate of the toxicity rate at each dose-level is provided. In model-based designs, the RP2D is defined as the dose that induces toxicity at the pre-defined target toxicity rate (mostly set to 10-33%) with an acceptable confidence interval according to the model[3]. Based on simulations, model-based designs are considered to establish the RP2D faster and more accurate while less patients are needed[1, 2, 5, 6, 9]. Additionally, it has been suggested that model-based designs are better when no expected RP2D can be pre-specified[1]. This is the case in many first-in-man trials and in drug-combination trials.

Also, there are model-based designs that allow incorporation of pharmacodynamic endpoints next to toxicity endpoints which is considered beneficial for molecularly targeted agents[1, 5]. Yet, the implementation of model-based designs in practice seems to be difficult. Their use may be hindered by insufficient statistical expertise and lack of familiarity compared to rule-based designs. In table 1, an overview of the theoretical advantages and drawbacks of the different designs for phase I trials is provided. In this systematic review, we aim to provide the true advantages and drawbacks of model-based and rule-based trials based on their actual performance.

## METHODS

**Table 1.** Comparison of the theoretical advantages and drawbacks of model-based and rule-based designs[1]. *RP2D , recommended phase II dose.*

| | Model-based | Rule-based |
|---|---|---|
| **Toxicity rate at RP2D** | Target rate to be specified, generally between 10 and 33% | Less than 33% |
| **Precision of RP2D** | Provides a confidence interval around selected RP2D | Uncertain |
| **Population size** | Likely to be smaller than rule-based | Likely to be larger than model-based |
| **Trial duration** | Likely to be short | Likely to be long |
| **Suboptimal dosing** | Likely to be minimal | Likely to be high |
| **Use of available information** | All clinical information incorporated in model for dose-escalation and for determining the RP2D | Only information of previous dose-level used during dose-escalation |
| **Implementation** | Statistical expertise needed | Easy to implement |
| **Application** | If no prior information on dose is available | If dose-levels can rationally be pre-specified |

### Search strategy

A search in PubMed was performed on June 28th 2014 to include all publications of phase I studies over the last 24 months addressing small-molecule targeted therapies and dose-escalation. The following search terms were used: ((((((maximum tolerated dose[mesh] OR maximum tolerated dos*[tiab] OR dose escalation*[tiab] OR doses escalation*[tiab] OR drug administration schedule[mesh] OR drug dose-response relationship[mesh])))) **AND** ((((molecular targeted therapy[mesh] OR targeted therap*[tiab] OR molecularly targeted therap*[tiab] OR inhibitor [tiab] OR small molecule*[tiab] OR tyrosine kinase*[tiab] OR kinase*[tiab] OR protein-tyrosine kinases[mesh])))))) **NOT** ((pediatric study[tiab] OR pediatric studies[tiab] OR pediatric[tiab] OR hormone therap*[tiab] OR hormonal therap*[tiab] OR radiotherap*[tiab] OR radio-therap*[tiab] OR cytotox*[tiab] OR children[tiab] OR virus[tiab] OR viral[tiab])) **AND** (phase I[tiab] OR phase 1[tiab] OR phase one[tiab] OR phase 1a[tiab] OR phase 1b[tiab] OR phase Ia[tiab] OR phase Ib[tiab]) **NOT** (expansion OR expansion phase) **Limits:** English, From: 2012/06/01 to 2014/06/01.

Search results were screened to include studies in which at least one small molecule targeted agent was escalated, either or not combined with fixed conventional chemotherapy/cytotoxic therapy. The following articles were excluded: paediatric studies, studies without dose-escalation/non-phase I studies, immunoglobulin therapies, gene therapy, vaccine/viral therapy, (combinations with) radiotherapy, non-oncologic applications and studies in which primary data were incomplete or inaccessible and early termination for reasons other than results on efficacy or tolerability. For each excluded trial, the principal reason for exclusion was recorded. Included articles were grouped by rule-based designs (key words: 3+3 or variants, mFibonacci escalation, accelerated titration, pharmacologically guided dose escalation (PGDE)) or model- based designs (key words: Bayesian model, continual reassessment model (CRM), escalation with overdose control (EWOC), toxicity probability method, nonparametric up and down design).

### Endpoints

Study characteristics were recorded including the PubMed identification number (PMID), the number of schedules that were tested, the number of escalations, reports on trial delay or amendments, the use of intermediate dose-levels, the number of active agents used in the trial, route of administration and first-in-man administration. No formal review protocol was used. Data on the endpoints as described in table 2 were extracted in duplicate by the first author. The median and range were reported and compared between designs.

Data on trial duration were obtained from ClinicalTrials.gov or the published article. If data on duration could not be retrieved authors were approached to complete data. From a random selection the authors were asked to report the trial duration in addition to published data to check for consistency. The overall toxicity rate was calculated for each trial based on the incidence of DLTs.

**Statistical analysis**
Data on all endpoints were compared between model-based and rule-based trials using a Wilcoxon rank-sum test with continuity correction. The correlation between trial duration and the number of included patients was assessed using Spearman's rank correlation (rho). Statistical tests were performed in R[13]. Subgroup analyses were performed for first-in-man studies, combination studies and studies with oral administration only, since it was expected that these factors may influence trial performance. Study characteristics were analyzed using Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables.

**Table 2.** Description of endpoints. *RP2D, recommended phase II dose; DLTs, dose-limiting toxicities; N, included population.*

| Endpoint | Description | Indicator of |
|---|---|---|
| Number of patients needed to establish RP2D | Number of patients receiving study treatment until the preliminary RP2D was identified and the cohort was expanded (i.e. for a 3+3 design when the cohort was expanded to more than six patients as formally needed to determine the RP2D) | Efficiency |
| Number of patients included | Number of patients enrolled/included | Efficiency |
| Number of escalations | As reported in publication | Efficiency |
| Ratio RP2D/starting dose | In case more than one schedule was tested, the first RP2D was divided by the initial starting dose. Starting dose refers to the starting point of dose-escalation (dose-level 1). | Efficiency |
| Trial duration | The time in months from start of the trial to data-closure as stated on ClinicalTrials.gov, the published article or as reported by the author | Efficiency |
| Patients treated below and at or above RP2D | Number of patients categorized per group that were treated below, and at or above RP2D as a percentage of the included population. The sum of patients treated below and at or above the RP2D may be lower than 100% in case the included population was larger than the number of treated patients | Patient safety<br>(Sub)optimal treatment |
| Number of DLTs | As reported in publication.<br>Toxicity rate in percentage for each trial was calculated by DLTs/ N included x 100% | Patient safety |

## RESULTS

Of the 343 search results, 171 publications were excluded for reasons as specified in figure 1 which left 172 studies for inclusion. Study characteristics and results on the pre-defined endpoints are presented in table 3. The complete overview of outcomes per included trial is available as Supplementary Material. Results on trial duration were obtained from 122 out of 172 trials, among these 23 were reported to us by the author. In total 68 authors were approached. Only the subgroup of first-in-man studies performed differently compared to the total dataset which will be discussed later.

**Performance of rule-based trials**
Among 172 trials that were included in this review, 161 (94%) used a rule-based design. All rule-based trials applied the 3+3 design or its variations and in 12 trials this was proceeded by an accelerated titration phase. The median time to finish was 36 months for rule-based trials with a median inclusion of 30 patients. Among the included population, 40% was treated at doses below the RP2D which is potentially suboptimal and 53% at the RP2D or above the RP2D which is potentially toxic. The starting dose was increased a median of 3.0 times in a median of 4 dose-escalations. For the dose-escalation part of the trials, a median of 26 patients was needed. In rule-based trials, the median number of DLTs was 3, which resulted in an incidence of DLTs (toxicity rate) of

on average 14%. This confirms that the toxicity rate in rule-based trials is indeed much lower than 33%, as has been suggested by Zhou *et al.*[11]. These numbers provide an indication of the performance of classical phase I trials but can only be interpreted when they are placed into perspective. Therefore, we provide a comparison to the performance of model-based trials.

**Performance of model-based trials**

In line with previous reviews, only 11 out of 172 trials that have been published in the last two years (6%) used a model-based design[3]. Of these, seven used a Bayesian Logistic Regression Model with Overdose Control (BLRM-EWOC), the others used BLRM, TITE-CRM, toxicity probability method or non-parametric up and down design with bivariate isotonic regression. The median time to finish was 26 months for model-based trials, with a median inclusion of 56 patients. Among these patients, 31% was treated below the RP2D and 60% at or above the RP2D. This confirms that model-based trials tend to treat more patients at or above the RP2D, as simulations suggested. Hereby, suboptimal treatment can be reduced. In model-based trials, the starting dose was increased median 2.0 times in a median of 6 dose-escalations. For the dose-escalation part of the trial, a median of 34 patients was needed. There was a large difference between the included population, consisting of 56 patients, and the population that was used for dose-escalation, consisting of 34 patients. This implies inclusion of more patients at or around the preliminary established RP2D. This may explain why in model-based trials, more patients were treated at or above the RP2D compared to rule-based trials. In model-based trials, the median number of DLTs was 5 per trial, which is slightly more than in rule-based trials. However, the total incidence of DLTs (toxicity rate) of 13% is comparable to the toxicity rate of 14% in rule-based trials.

**Clinically relevant differences in trial duration and population size**

Although we were not able to detect any significant differences in the operating characteristics for trial performance (table 3), we observed pronounced differences in trial duration (36 vs. 26 months; $p$=0.25) and population size (30 vs. 56 patients; $p$=0.09), which are considered clinically relevant. Paradoxically, whereas the data on trial duration favor model-based trials, the data on population size seem to favor rule-based trials. In the next paragraphs, we will discuss possible explanations for these findings.

**Trial delay**

The median time to finish data collection was 26 months (range: 16-48 months) for model based trials, whereas rule-based trials needed 36 months (range: 8-90 months). Despite the wide ranges in trial duration in both groups, a clear trend towards shorter duration of model-based trials is visible with a median difference of 10 months (figure 2).

Several factors could contribute to prolonged trial duration. These include the investigation of more dose-levels, a high ratio between the starting dose and the RP2D, inclusion of more patients, or a high incidence of DLTs during the trial. However, rule-based trials did not investigate more dose-levels, nor did they perform more escalations, report more DLTs or include more patients than model-based trials. Paradoxically, the number of included patients was even lower in rule-based trials. These parameters could not explain why rule-based trials needed more time to finish. Therefore, we explored other factors that could contribute to the difference in trial duration. We found that in 43% of the rule-based trials, trial delay was reported whereas this was the case in none of the model-based trials. Reasons for trial delay included amendments to the protocol (18%) or introduction of intermediate dose-levels (39%). Rule-based trials for which such a delay was reported lasted 41 months, whereas those for which no delay was reported lasted only 31 months ($p$=0.02).

**More patients in first-in-man model-based trials**

Although we expected that shorter trial duration of model-based trials would coincide with a smaller population size, this was not the case. Trial duration was weakly associated with sample size (Spearman's rho=0.26, $p$=0.004) and while model-based trials took 10 months less to finish, they accrued 46% more patients (30 for rule-based trials vs. 56 for model-based trials; $p$=0.09).

It may be noticed that the presented data on population size in model-based trials show a discrepancy. Model-based trials included more patients than rule-based trials while only slightly more patients were needed to determine the RP2D. This could be explained by the inclusion of more patients at or around the preliminary established RP2D in model-based trials. Another possible explanation could come from the first-in-man studies, which may contribute to the larger population sizes in model-based trials. Within the first-in-man studies, model-based trials (n=3) did not only include more patients (median 101 vs. 44; $p$=0.03) but also needed significantly more patients to determine the preliminary RP2D (median 75 vs. 34; $p$=0.02) compared to rule-based (n=55) trials. Additionally, the first-in-man model-based trials had a remarkably high ratio between the RP2D and the starting dose (median 35.0 for model-based vs. 8.0 for rule-based; $p$=0.11). In this subgroup, the median

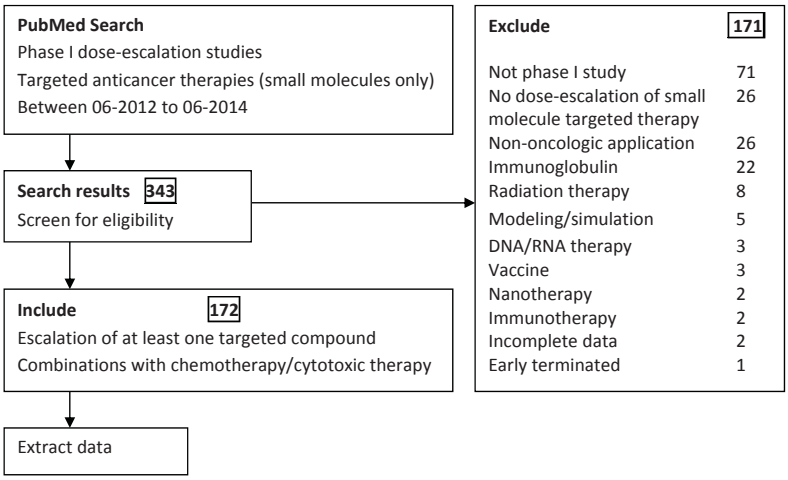


**Figure 1.** Study selection overview

**Table 3.** Characteristics and performance of rule-based trials versus model-based trials.

| **Trials included** | **Rule-based** 161 (100%) | **Model-based** 11 (100%) | ***p* value** |
|---|---|---|---|
| First in man (FIM) studies | 55 (34%) | 3 (27%) | 0.75* |
| Combination therapies (including 4 FIM studies) | 66 (41%) | 3 (27%) | 0.53* |
| Schedules tested | | | 0.87^ |
| *1* | 107 (66%) | 7 (64%) | |
| *2* | 37 (23%) | 3 (27%) | |
| *≥3* | 17 (11%) | 1 (9.0%) | |
| Administration route oral | 117 (73%) | 9 (82%) | 0.73* |
| Number of patients needed to determine RP2D[b] | 26 [8-147] | 34 [15-135] | 0.07^ |
| Number of patients[b] | 30 [8-206] | 56 [15-135] | 0.09^ |
| Patients treated below RP2D (% of included)[b] | 40 [0-100] | 31 [0-68] | 0.73^ |
| Patients treated at or above RP2D (% of included)[b] | 53 [0-100] | 60 [21-100] | 0.76^ |
| Number of DLTs in the trial[b] | 3 [0-18] | 5 [1-28] | 0.14^ |
| Number of escalations[b] | 4 [0-20] | 6 [1-12] | 0.55^ |
| Ratio RP2D/starting dose[b] | 3.0 [0-180] | 2.0 [1-40] | 0.96^ |
| Trial duration[a] in months[b] | 36 [8-90] | 26 [16-48] | 0.25^ |

[a] Available for 113 (70%) rule-based and 9 (82%) model-based studies
[b] Values presented as: median [range]
* Obtained by Fisher's Exact Test; ^ Obtained by Wilcoxon rank-sum test

difference between trial duration of model-based trials and rule-based trials decreased to 4 months (31 vs. 35 months; $p$=0.9) instead of 10 months. Although data are limited, this raises the question whether model-based trials are less suitable for first-in-man studies, which we will discuss below.

**Increased accuracy of RP2D**

In the previous paragraph we discussed the finding that especially in first-in-man trials, model-based trials included more patients and that these trials lasted only marginally shorter. This can be interpreted in two ways. Firstly, it may indicate that the use of a model-based design leads to inferior performance of first-in-man trials, but this would be difficult to explain. Secondly, it may be an indication that where a rule-based design would stop too early, a model-based design could continue and establish a more accurate RP2D. Such a scenario would be likely in particular for first-in-man studies, where no clinical information is available in advance to predict the RP2D. As a result, model-based and rule-based designs may find very different RP2Ds in first-in-man trials. This was confirmed in practice in the first-in-man model-based study by Sessa *et al*.[14]. They reported that the RP2D they found was 2.5 times higher than the RP2D as it would have been defined by a rule-based approach. More data should be obtained to investigate if model-based trials indeed need more patients and if this results in a more accurate estimate of the RP2D.

**Quicker estimate of the RP2D in model-based trials**

In line with the shorter trial duration, it seems that model-based trials are able to provide a first estimate of the RP2D more swiftly. To establish the RP2D, model-based trials investigated more dose-levels (6 vs. 4; $p$ = 0.55) and needed more patients (34 vs. 26; $p$ = 0.07). Yet, the total trial duration was shorter.

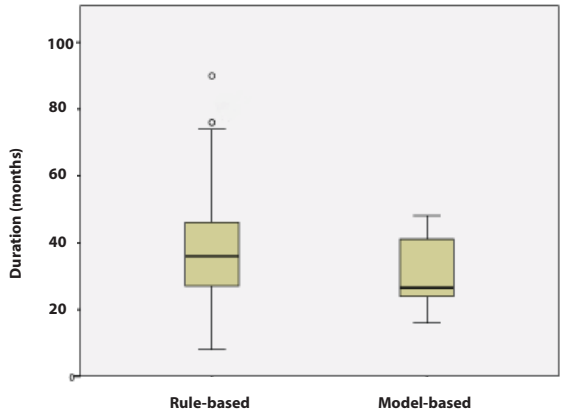


**Figure 2.** Trial duration of rule-based versus model-based trials.

Additionally, model-based trials approach the RP2D rather quick given the low percentage of patients that were treated at levels below the RP2D (31% vs. 40%; $p$=0.73). Quicker dose-escalation does not seem to have compromised patient safety, since the toxicity rate of model-based trials (13%) and rule-based trials (14%) was comparable. It can thus be stated that the swift dose-escalation in model-based trials does not affect patient safety.

## DISCUSSION AND CONCLUSION

Among the 172 included phase I studies that were published between 2012 and 2014 only 11 used a model-based design. Although we expected that more eligible model-based trials could be included over the last two years, the included number fits with previous reviews[3]. The relatively low number of model-based trials reflects current practice; a slight trend towards the use of model-based designs is noticed, but the use of rule-based designs is prevailing persuasively. Despite the low number, the data we report on model-based trials are comparable to the data that were reported by Iasonos *et al.* in a review based on 53 model-based designs and are therefore considered representative[3].

With the presented data we tried to answer the question whether or not there is evidence to prefer a model-based design over a rule-based design. Based on results from simulations, model-based designs have been considered superior to rule-based designs. We provided data on trial performance in practice to allow comparison of performance of model-based and rule-based trials. We found no statistical superiority of either rule-based trials or model-based trials. However, our data suggest that with model-based designs, the RP2D can be established more swiftly compared to rule-based trials. Additionally, we showed that patients are more likely to receive optimal and potentially effective doses in a model-based phase I trial without additional severe toxicity. This has been assumed before but was never truly compared to rule-based trials[1, 5]. A disadvantage of model-based trials is that more patients are needed overall, but this may be counterbalanced by a more accurate estimate of the RP2D.

The evidence we provide is limited by several factors. Firstly, we retrieved data on trial duration from three sources but these data were highly inconsistent. We searched ClinicalTrials.gov, extracted data on duration from the published articles and additionally we asked authors to report the duration of their trial. If available, data from ClinicalTrials.gov or the published article were used. Otherwise the authors' report was used. Ideally, trial duration should have been defined as the time from start of accrual to determination of the RP2D, but since these data were not available we have defined it as time from start to data closure.

Secondly, only few publications on model-based trials could be included. Although our search was broadened to increase the number of model-based trials, the proportion of model-based trials remained low. This should be considered when interpreting the results. Selective publication could have biased our results. However, there is no reason nor evidence to assume that either model-based or rule-based trials are more prone to selective publication.

Thirdly, the performance of individual trials can be affected by several factors that are not included in this analysis, such as the investigational product, speed of recruitment, the number of participating centers, financial and logistical issues. Heterogeneity of the included trials possibly contributes to the wide variation in the data and to non-significant differences between designs. Additionally, a crucial aspect of phase I trial performance is the accuracy of the RP2D. However, this is difficult to address because for many trials it is unknown what RP2D would have been found if another design was used. Despite these limitations, we encountered strong indications that establishment of the RP2D with model-based designs is quick and safe.

For future phase I trials we encourage the use of model-based designs in order to shorten clinical development of anticancer agents and to potentially increase patient benefit. Currently, dose-escalation trials use toxicity data (DLTs) as the primary endpoint. Since the introduction of targeted anticancer agents and immunotherapy, the use of toxicity data as the only endpoint has become doubtful. There is an increasing need for additional endpoints, such as pharmacodynamics, to support the optimal dose. Model-based designs allow using pharmacodynamic data next to toxicity data[1, 5, 10], whereas in rule-based designs the use of different outcome parameters is problematic. Current research on biomarker development and validation will hopefully facilitate incorporation of pharmacodynamic endpoints in dose-escalation studies. Although previously FDA, EMA, and several reviewers already recommended the use of model-based designs[1, 2, 5, 6], the use is still uncommon. The implementation of model-based designs into daily practice may be hindered by the lack of familiarity with these designs and insufficient statistical expertise. We hope these obstacles may be overcome to improve the performance of dose-escalation trials. Pharmaceutical companies, patients, and society may benefit from the use of model-based trials given their potential to shorten clinical development of novel therapies.

**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

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**SUPPLEMENTARY MATERIAL - OUTCOMES OF INCLUDED TRIALS**

**Table 1**. Data on pre-defined endpoints and operating characteristics per individual trial. *N, number of patients; RP2D, recommended phase 2 dose; DLT, dose-limiting toxicity; iv, intravenously.*

| PubMed Identification Number (PMID) | Author | Duration (months) | N needed to determine RP2D | N enrolled | Below RP2D (%) | At or above RP2D (%) | DLTs | Escalations | Ratio RP2D/Starting dose | First-in-man (1=yes 2=no) | Monotherapy (1) or combination (2) | Schedules | Administration (1=iv 2=oral 3= other) | Toxicity rate (%) | Report on delay (1=yes 2= no) | Intermediate dose levels introduced | Design (1=rule-based 2= model-based) | LRM = Logistic regression model<br>CRM= continuous reassessment model<br>TITE = time to event |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24879333 | Falchook et al. | 22 | 24 | 24 | 33.3 | 66.7 | 1 | 4 | 5 | 2 | 1 | 1 | 2 | 4.2 | 1 | 3 | 1 | 3+3 |
| 24849582 | Mita et al. | | 97 | 97 | 100 | 0 | 2 | 6 | 8 | 2 | 2 | 5 | 2 | 2.1 | 2 | 1 | 1 | 3+3 |
| 24807459 | Horinouchi et al. | 21 | 12 | 12 | 50 | 50 | 2 | 1 | 1.7 | 2 | 2 | 1 | 2 | 16.7 | 2 | 2 | 1 | 6+6 |
| 24755882 | Lin et al. | 26 | 32 | 59 | 5.1 | 23.7 | 6 | 4 | 3 | 2 | 1 | 2 | 1 | 10.2 | 2 | 2 | 1 | 3+3 |
| 24711549 | Chien et al. | | 19 | 19 | 78.9 | 21.1 | 0 | 5 | 4 | 2 | 1 | 2 | 2 | 0 | 2 | 1 | 1 | 3+3 |
| 24663045 | Mayer et al. | 25 | 51 | 51 | 0 | 100 | 3 | 0 | 1 | 2 | 2 | 2 | 2 | 5.9 | 2 | 2 | 1 | 3+3 |
| 24636848 | Reguart et al. | | 14 | 14 | 42.9 | 42.9 | 1 | 2 | 1.4 | 2 | 2 | 2 | 2 | 7.1 | 2 | 2 | 1 | 3+3 |
| 24612546 | Zhang et al. | | 42 | 71 | 66.2 | 33.8 | 5 | 10 | 7.5 | 1 | 1 | 2 | 2 | 7 | 2 | 2 | 1 | 3+3 |
| 24583798 | Papadopoulos et al. | 64 | 53 | 83 | 18.1 | 81.9 | 9 | 6 | 3.3 | 1 | 1 | 2 | 2 | 10.8 | 2 | 1 | 1 | 3+3 |
| 24577167 | Ganesan et al. | 39 | 22 | 31 | 29 | 71 | 6 | 5 | 1 | 2 | 2 | 1 | 2 | 19.4 | 1 | 1 | 1 | 3+3 |
| 24523439 | Rodon et al. | 31 | 75 | 103 | 16.5 | 83.5 | 19 | 9 | 8 | 1 | 1 | 2 | 2 | 18.4 | 2 | 2 | 2 | Bayesian LRM with overdose control |
| 24496004 | Janne et al. | 30 | 46 | 46 | 39.1 | 60.9 | 4 | 5 | 2.3 | 2 | 2 | 2 | 2 | 8.7 | 2 | 2 | 1 | 3+3 |
| 24493827 | Bowles et al. | | 18 | 18 | 44.4 | 55.6 | 2 | 4 | 8 | 1 | 1 | 1 | 2 | 11.1 | 2 | 2 | 1 | Accelerated titration followed by 3+3 |
| 24470557 | Wen et al. | 60 | 15 | 22 | 0 | 95.5 | 12 | 2 | 3.3 | 2 | 2 | 1 | 2 | 54.5 | 1 | 1 | 1 | 3+3 |
| 24470511 | Saura et al. | | 18 | 18 | 27.8 | 66.7 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 5.6 | 2 | 2 | 2 | Bayesian LRM with overdose control |
| 24456794 | Hollebecque et al. | 16 | 29 | 29 | 0 | 100 | 4 | 5 | 0 | 2 | 2 | 2 | 2 | 13.8 | 2 | 1 | 1 | 3+3 |
| 24448638 | Sausville et al. | 41 | 42 | 42 | 50 | 50 | 5 | 6 | 5 | 1 | 2 | 2 | 1 | 11.9 | 1 | 1 | 1 | 3+3 |
| 24440085 | Tolcher et al. | 41 | 34 | 34 | 41.2 | 58.8 | 7 | 7 | 0.7 | 1 | 1 | 2 | 2 | 20.6 | 1 | 1 | 1 | 3+3 |
| 24429877 | Gray et al. | 57 | 18 | 18 | 16.7 | 83.3 | 3 | 2 | 1.1 | 2 | 2 | 1 | 2 | 16.7 | 2 | 2 | 1 | 3+3 |
| 24405565 | Ando et al. | 24 | 15 | 15 | 40 | 60 | 1 | 2 | 4 | 2 | 1 | 1 | 2 | 6.7 | 2 | 2 | 2 | Bayesian LRM with overdose control |
| 24366297 | Hamberg et al. | | 49 | 61 | 4.9 | 77 | 11 | 4 | 2 | 2 | 2 | 2 | 2 | 18 | 2 | 2 | 1 | 3+3+3 |
| 24352795 | Kelly et al. | 59 | 58 | 58 | 36.2 | 60.3 | 11 | 9 | 4 | 2 | 1 | 4 | 2 | 19 | 1 | 1 | 1 | 3+3 |
| 24323026 | Gandhi et al. | 26 | 64 | 64 | 67.2 | 26.6 | 10 | 11 | 1.7 | 2 | 2 | 1 | 2 | 15.6 | 2 | 2 | 4 | Non-parametric up and down design with bivariate isotonic regression |
| 24252402 | Hudis et al. | 23 | 34 | 34 | 8.8 | 82.4 | 5 | 3 | 1.3 | 2 | 2 | 2 | 2 | 14.7 | 2 | 2 | 4 | Toxicity probability interval method |
| 24241210 | Hofmeister et al. | 62 | 15 | 15 | 53.3 | 46.7 | 0 | 2 | 1.7 | 2 | 1 | 1 | 1 | 0 | 2 | 2 | 1 | 3+3 |
| 24166903 | Shapiro et al. | 52 | 38 | 69 | 42 | 62.3 | 4 | 9 | 20 | 1 | 1 | 2 | 2 | 5.8 | 1 | 2 | 1 | 3+3 |
| 24149182 | Adenis et al. | 27 | 12 | 12 | 66.7 | 33.3 | 0 | 2 | 2 | 2 | 2 | 1 | 2 | 0 | 2 | 3 | 1 | 3+3 |
| 24131779 | Nemunaitis et al. | 74 | 52 | 52 | 61.5 | 30.8 | 5 | 10 | 36.4 | 2 | 1 | 1 | 1 | 9.6 | 2 | 2 | 1 | Accelerated titration |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24077916 | Jankowitz et al. | 16 | 21 | 21 | 57.1 | 42.9 | 4 | 3 | 1.7 | 2 | 2 | 1 | 2 | 19 | 2 | 1 | 1 | 3+3 |
| 24072436 | Dent et al. | 60 | 35 | 35 | 62.9 | 37.1 | 2 | 8 | 2.7 | 2 | 1 | 1 | 1 | 5.7 | 2 | 1 | 1 | 3+3 |
| 24057042 | Gadgeel et al. | | 26 | 26 | 34.6 | 50 | 6 | 5 | 1.7 | 2 | 2 | 1 | 2 | 23.1 | 2 | 2 | 1 | 3+3 |
| 24043137 | Zhou et al. | 43 | 55 | 55 | 69.1 | 30.9 | 2 | 7 | 6.3 | 1 | 1 | 1 | 2 | 3.6 | 2 | 1 | 1 | 3+3 |
| 24033250 | Muller-Tidow et al. | 36 | 59 | 78 | 15.4 | 50 | 10 | 8 | 1.8 | 2 | 1 | 3 | 1 | 12.8 | 1 | 1 | 1 | 3+3 |
| 24002496 | Cortes et al. | 26 | 76 | 76 | 67.1 | 32.9 | 15 | 9 | 16.7 | 1 | 1 | 2 | 2 | 19.7 | 2 | 1 | 1 | 3+3 |
| 23963360 | Wheler et al. | | 20 | 20 | 10 | 90 | 3 | 1 | 1.5 | 2 | 2 | 1 | 2 | 15 | 2 | 2 | 1 | 3+3 |
| 23962904 | Mansfield et al. | 47 | 15 | 15 | 40 | 60 | 2 | 2 | 2 | 2 | 1 | 1 | 3 | 13.3 | 2 | 2 | 1 | 3+3 |
| 23959599 | Jones et al. | 62 | 46 | 46 | 100 | 0 | 0 | 2 | 0 | 2 | 1 | 1 | 1 | 0 | 2 | 2 | 1 | 3+3 |
| 23942080 | Bowles et al. | 39 | 26 | 43 | 46.5 | 53.5 | 0 | 2 | 2 | 2 | 2 | 1 | 2 | 0 | 2 | 2 | 1 | 6+3 |
| 23887852 | Kumar et al. | 53 | 14 | 14 | 57.1 | 42.9 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 14.3 | 2 | 2 | 1 | 3+3 |
| 23873691 | Hong et al. | 28 | 29 | 45 | 40 | 51.1 | 2 | 6 | 24 | 1 | 1 | 1 | 1 | 4.4 | 1 | 1 | 1 | 3+3 |
| 23868188 | Mayer et al. | | 18 | 18 | 61.1 | 38.9 | 2 | 2 | 3 | 2 | 2 | 1 | 2 | 11.1 | 2 | 2 | 1 | 3+3 |
| 23860959 | Honda et al. | | 12 | 12 | 75 | 25 | 0 | 3 | 2.8 | 2 | 1 | 1 | 1 | 0 | 2 | 2 | 1 | 3+3 |
| 23857959 | Kelly et al. | 34 | 22 | 22 | 54.5 | 45.5 | 3 | 5 | 2.8 | 2 | 2 | 1 | 1 | 13.6 | 2 | 2 | 1 | 3+3 |
| 23847256 | Plimack et al. | 29 | 38 | 38 | 60.5 | 39.5 | 4 | 7 | 0 | 1 | 1 | 2 | 2 | 10.5 | 2 | 1 | 1 | 3+3 |
| 23832397 | Mita et al. | 46 | 92 | 92 | 48.9 | 19.6 | 7 | 15 | 12.3 | 1 | 1 | 3 | 2 | 7.6 | 1 | 1 | 1 | 3+3 |
| 23817974 | LoRusso et al. | 46 | 43 | 54 | 55.56 | 44.4 | 14 | 7 | 8 | 2 | 1 | 1 | 2 | 25.9 | 2 | 1 | 1 | 3+3 |
| 23810467 | Liu et al. | | 17 | 28 | 21.4 | 78.6 | 2 | 3 | 2 | 2 | 2 | 1 | 2 | 7.1 | 2 | 2 | 1 | 3+3 |
| 23789936 | Komrokji et al. | | 15 | 37 | 18.9 | 81.0 | 2 | 4 | 8 | 2 | 1 | 3 | 2 | 5.4 | 2 | 2 | 1 | Accelerated titration followed by 3+3 |
| 23763917 | Kantarjian et al. | 41 | 32 | 32 | 62.5 | 37.5 | 4 | 6 | 24 | 2 | 1 | 1 | 2 | 12.5 | 2 | 2 | 1 | 3+3 |
| 23679664 | Niwakawa et al. | 16 | 9 | 9 | 33.3 | 66.7 | 0 | 1 | 1.5 | 2 | 1 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 23639470 | Seto et al. | 23 | 24 | 24 | 50 | 50 | 0 | 7 | 15 | 2 | 1 | 2 | 2 | 0 | 1 | 2 | 1 | Accelerated titration followed by 3+3 |
| 23635552 | Govindan et al. | 26 | 19 | 19 | 0 | 100 | 4 | 2 | 0.7 | 2 | 2 | 1 | 2 | 21.1 | 2 | 2 | 1 | 3+3 |
| 23632474 | Chow et al. | | 8 | 8 | 37.5 | 62.5 | 0 | 1 | 1.5 | 2 | 2 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 23614653 | Hainsworth et al. | | 9 | 9 | 0 | 100 | 1 | 4 | 0.5 | 2 | 2 | 1 | 2 | 11.1 | 2 | 1 | 1 | 3+3 |
| 23539344 | Raymond et al. | 35 | 54 | 66 | 57.6 | 42.4 | 9 | 10 | 3.7 | 2 | 2 | 3 | 1 | 13.6 | 2 | 3 | 1 | 3+3 |
| 23490650 | Soria et al. | 35 | 28 | 28 | 42.9 | 57.1 | 4 | 5 | 5 | 1 | 1 | 1 | 2 | 14.3 | 2 | 1 | 1 | 3+3 |
| 23434733 | Weekes et al. | | 49 | 49 | 67.3 | 32.7 | 8 | 9 | 50 | 1 | 1 | 1 | 2 | 16.3 | 2 | 2 | 1 | 3+3 |
| 23433846 | Cohen et al. | 43 | 73 | 82 | 74.4 | 25.6 | 4 | 10 | 80 | 1 | 1 | 2 | 2 | 4.9 | 2 | 1 | 1 | 6+3 |
| 23361621 | Aung Naing et al. | | 44 | 44 | 34.1 | 65.9 | 10 | 8 | 20 | 1 | 1 | 1 | 2 | 22.7 | 2 | 1 | 1 | 3+3 |
| 23339124 | Angevin et al. | 48 | 16 | 20 | 0 | 100 | 4 | 1 | 1 | 2 | 1 | 1 | 2 | 20 | 2 | 2 | 2 | Bayesian LRM with overdose control |
| 23228190 | Mayr et al. | 38 | 35 | 35 | 34.3 | 37.1 | 3 | 4 | 2 | 2 | 2 | 2 | 2 | 8.6 | 2 | 2 | 1 | 3+3 |
| 23190221 | Cortes et al. | | 81 | 81 | 38.3 | 61.7 | 7 | 6 | 22.5 | 1 | 1 | 1 | 2 | 8.6 | 2 | 2 | 1 | 3+3 |
| 23178951 | Tibes et al. | 19 | 22 | 22 | 86.4 | 13.6 | 0 | 9 | 15 | 1 | 1 | 1 | 2 | 0 | 2 | 1 | 1 | Accelerated titration followed by 3+3 |
| 23146956 | Simonelli et al. | | 18 | 30 | 40 | 53.3 | 2 | 3 | 1.7 | 2 | 2 | 1 | 2 | 6.7 | 2 | 2 | 1 | 3+3 |
| 23108698 | Michael et al. | 22 | 10 | 10 | 0 | 100 | 3 | 1 | 1 | 2 | 2 | 1 | 2 | 30 | 2 | 2 | 1 | 3+3 |
| 23099652 | Kreisl et al. | | 15 | 15 | 20 | 80 | 3 | 2 | 1.3 | 2 | 1 | 1 | 2 | 20 | 2 | 2 | 1 | 3+3 |
| 23099651 | Lee et al. | | 13 | 13 | 53.8 | 46.2 | 4 | 1 | 2 | 2 | 2 | 1 | 2 | 30.8 | 2 | 2 | 1 | 3+3 |
| 23099006 | Macpherson et al. | 29 | 21 | 21 | 33.3 | 66.7 | 3 | 2 | 1 | 2 | 2 | 1 | 2 | 14.3 | 2 | 2 | 1 | 3+3 |
| 23014528 | Baselga et al. | 54 | 72 | 72 | 80.6 | 73.6 | 5 | 9 | 16 | 1 | 1 | 1 | 2 | 6.9 | 2 | 1 | 1 | 3+3 |
| 23001754 | Mayer et al. | 13 | 23 | 23 | 34.8 | 65.2 | 4 | 2 | 2 | 2 | 2 | 1 | 2 | 17.4 | 2 | 2 | 1 | 8+8 |
| 22978685 | Vose et al. | 37 | 48 | 48 | 47.9 | 39.6 | 6 | 8 | 3.5 | 2 | 1 | 1 | 1 | 12.5 | 2 | 1 | 1 | 3+3 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22968184 | Papadimitrakopoulou et al. | | 94 | 94 | 76.6 | 23.4 | 18 | 7 | 1.5 | 2 | 2 | 2 | 2 | 19.1 | 2 | 1 | 1 | 6+6 |
| 22967996 | Awada et al. | | 12 | 12 | 50 | 50 | 1 | 1 | 1.5 | 2 | 2 | 1 | 2 | 8.3 | 2 | 2 | 1 | 3+3 |
| 22965964 | Younes et al. | 39 | 34 | 34 | 64.7 | 55.9 | 3 | 4 | 4 | 1 | 1 | 1 | 2 | 8.8 | 2 | 3 | 1 | 3+3 |
| 22921184 | Mahadevan et al. | 45 | 44 | 44 | 88.6 | 11.4 | 1 | 8 | 12.3 | 1 | 1 | 1 | 1 | 2.3 | 2 | 2 | 1 | 3+3 |
| 22878519 | Nokihara et al. | 8 | 9 | 9 | 33.3 | 66.7 | 0 | 1 | 2 | 2 | 1 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 22872523 | De Boer et al. | | 34 | 46 | 19.6 | 78.3 | 3 | 4 | 2.5 | 2 | 2 | 2 | 2 | 6.5 | 2 | 2 | 1 | 4+2 |
| 22805291 | Infante et al. | 35 | 55 | 206 | 9.7 | 88.3 | 5 | 20 | 16 | 1 | 1 | 5 | 2 | 2.4 | 2 | 1 | 1 | Accelerated titration followed by 3+3 |
| 22767670 | Dees et al. | 39 | 73 | 73 | 37 | 52.1 | 11 | 13 | 20 | 1 | 1 | 3 | 2 | 15.1 | 2 | 2 | 1 | 3+3 |
| 22753585 | Cervantes et al. | 35 | 51 | 59 | 15.3 | 84.7 | 11 | 9 | 20 | 2 | 1 | 3 | 2 | 18.6 | 2 | 1 | 1 | 3+3 |
| 22701615 | Meyerhardt et al. | 60 | 17 | 27 | 25.9 | 74.1 | 2 | 3 | 2 | 2 | 2 | 1 | 2 | 7.4 | 1 | 2 | 1 | 3+3 |
| 22693357 | Hong et al. | 39 | 46 | 71 | 39.4 | 57.7 | 4 | 11 | 24 | 1 | 1 | 2 | 2 | 5.6 | 2 | 2 | 1 | 3+3 |
| 22661287 | Schwartz et al. | | 35 | 35 | 37.1 | 62.9 | 5 | 6 | 6 | 2 | 1 | 2 | 1 | 14.3 | 2 | 2 | 1 | 3+3 |
| 22634319 | Hong et al. | 44 | 28 | 47 | 42.6 | 72.3 | 3 | 9 | 180 | 1 | 1 | 1 | 2 | 6.4 | 2 | 1 | 1 | 3+3 |
| 22605616 | Goldman et al. | 30 | 18 | 32 | 9.38 | 90.6 | 2 | 4 | 3 | 2 | 2 | 1 | 2 | 6.3 | 2 | 2 | 1 | 8 with intrapatient dose escalation, then 3 + 3 |
| 22549288 | Beasley et al. | 22 | 16 | 20 | 0 | 100 | 4 | 1 | 1 | 2 | 2 | 1 | 2 | 20 | 2 | 2 | 1 | 2+4 |
| 22547164 | Infante et al. | 12 | 10 | 10 | 0 | 100 | 10 | 1 | 0 | 1 | 1 | 2 | 2 | 100 | 2 | 2 | 1 | 3+3 |
| 22524974 | Olnes et al. | 57 | 14 | 14 | 21.4 | 78.6 | 3 | 2 | 1.7 | 1 | 1 | 1 | 1 | 21.4 | 2 | 2 | 1 | 3+3 |
| 22492020 | Weiss et al. | 32 | 25 | 31 | 61.3 | 29 | 5 | 4 | 3.8 | 1 | 2 | 1 | 1 | 16.1 | 2 | 1 | 1 | 3+3 |
| 22448814 | Ogura et al. | 55 | 13 | 13 | 100 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 2 | 0 | 2 | 1 | 1 | 3+3 |
| 22415795 | Fujisaka et al. | 35 | 12 | 12 | 25 | 75 | 2 | 2 | 2.5 | 1 | 1 | 1 | 2 | 16.7 | 2 | 2 | 1 | 3+3 |
| 22407177 | Reardon et al. | 30 | 47 | 47 | 51.1 | 48.9 | 8 | 3 | 1.8 | 2 | 2 | 2 | 2 | 17 | 2 | 2 | 1 | 3+3 |
| 22382879 | Asahina et al. | 45 | 18 | 18 | 66.7 | 33.3 | 2 | 10 | 5 | 2 | 1 | 1 | 1 | 11.1 | 2 | 2 | 1 | 3+3 |
| 22357447 | Markman et al. | 26.5 | 57 | 57 | 57.9 | 42.1 | 3 | 6 | 40 | 1 | 1 | 1 | 2 | 5.3 | 2 | 2 | 2 | Bayesian LRM with overdose control |
| 22280537 | Wang et al. | 17 | 30 | 47 | 74.5 | 21.3 | 8 | 4 | 2 | 1 | 1 | 2 | 2 | 17 | 2 | 2 | 1 | 3+3 |
| 22249430 | Takahashi et al. | 28 | 13 | 13 | 46.2 | 53.8 | 0 | 2 | 3 | 2 | 1 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 22160853 | Weiss et al. | 37 | 38 | 38 | 23.7 | 73.7 | 10 | 6 | 3 | 2 | 1 | 2 | 2 | 26.3 | 1 | 3 | 1 | 3+3 |
| 21881918 | Argiris et al. | 25 | 19 | 31 | 9.7 | 90.3 | 3 | 2 | 1.5 | 2 | 2 | 1 | 2 | 9.7 | 2 | 2 | 1 | 3+3 |
| 21881915 | Bryce et al. | 36 | 35 | 48 | 25 | 41.7 | 10 | 6 | 1.6 | 2 | 2 | 2 | 3 | 20.8 | 1 | 1 | 1 | 3+3 |
| 21863237 | Ramanathan et al. | 14 | 10 | 14 | 21.4 | 78.6 | 2 | 2 | 1.3 | 2 | 1 | 1 | 1 | 14.3 | 2 | 2 | 1 | 3+3 |
| 21567184 | Shin et al. | | 11 | 11 | 0 | 100 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 18.2 | 2 | 2 | 1 | 3+3 |
| 24374145 | Verstovsek et al. | 24 | 30 | 30 | 0 | 100 | 0 | 5 | 0 | 1 | 1 | 3 | 2 | 0 | 2 | 1 | 1 | 3+3 |
| 24192770 | Martin et al. | | 13 | 13 | 0 | 100 | 4 | 1 | 1.3 | 2 | 2 | 1 | 2 | 30.8 | 2 | 2 | 1 | 3+3 |
| 24370210 | Luu et al. | 52 | 11 | 11 | 0 | 100 | 5 | 1 | | 2 | 2 | 1 | 3 | 45.5 | 2 | 1 | 1 | 3+3 |
| 24355079 | Foran et al. | 31 | 32 | 48 | 47.9 | 52.1 | 11 | 9 | 36 | 2 | 1 | 3 | 1 | 22.9 | 2 | 2 | 1 | 3+3 |
| 22767668 | Leijen et al. | 61 | 46 | 49 | 53.1 | 26.5 | 2 | 8 | 11.3 | 1 | 1 | 1 | 2 | 4.1 | 2 | 3 | 1 | 3+3 |
| 22683301 | Schimmer et al. | 24 | 13 | 13 | 37.5 | 76.9 | 2 | 4 | 3 | 1 | 1 | 1 | 2 | 15.4 | 2 | 2 | 1 | 3+3 |
| 22609229 | Felip et al. | | 15 | 15 | 40 | 60 | 0 | 1 | 1.5 | 2 | 2 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 24880570 | Plummer et al. | 28 | 23 | 26 | 46.2 | 53.8 | 4 | 4 | 5 | 1 | 2 | 1 | 2 | 15.4 | 2 | 2 | 1 | 3+3 |
| 24875132 | Kurata et al. | 30 | 17 | 27 | 11.1 | 51.9 | 0 | 2 | 1.5 | 2 | 3 | 2 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 24819685 | Bendell et al. | 41 | 27 | 30 | 46.7 | 53.3 | 3 | 5 | 6 | 1 | 1 | 1 | 2 | 10 | 2 | 2 | 1 | 3+3 |
| 24434430 | Bauer et al. | | 12 | 12 | 0 | 100 | 8 | 1 | 1.5 | 2 | 2 | 1 | 2 | 66.7 | 2 | 2 | 1 | 3+3 |
| 24413064 | Tan et al. | | 22 | 39 | 28.2 | 71.8 | 6 | 3 | 3 | 2 | 2 | 1 | 2 | 15.4 | 2 | 2 | 1 | 3+3 |
| 24138944 | Liesveld et al. | 32 | 13 | 13 | 0 | 100 | 0 | 2 | 1 | 2 | 2 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24097863 | Saif et al. | 29 | 49 | 60 | 53.3 | 40 | 2 | 10 | 24 | 1 | 1 | 2 | 2 | 3.3 | 2 | 2 | 1 | 3+3 |
| 24077982 | Hollebecque et al. | | 44 | 44 | 63.6 | 36.4 | 4 | 10 | 54 | 1 | 1 | 1 | 1 | 9.1 | 2 | 2 | 1 | 3+3 |
| 24065624 | Brunetto et al. | 25 | 19 | 19 | 47.4 | 52.6 | 1 | 4 | 6 | 1 | 1 | 1 | 2 | 5.3 | 2 | 1 | 1 | 3+3 |
| 24045182 | Wagner et al. | | 28 | 54 | 22.2 | 70.4 | 4 | 5 | 4.4 | 1 | 1 | 1 | 1 | 7.4 | 2 | 2 | 1 | 3+3 |
| 23983259 | Eskens et al. | | 30 | 40 | 50 | 50 | 2 | 6 | 6.7 | 1 | 1 | 1 | 1 | 5 | 2 | 1 | 1 | 3+3 |
| 23963141 | Sun et al. | 29 | 15 | 21 | 28.6 | 66.7 | 4 | 2 | 2 | 2 | 2 | 1 | 2 | 19 | 2 | 2 | 1 | 6+6 |
| 23930209 | Werner et al. | 55 | 12 | 16 | 37.5 | 62.5 | 1 | 2 | 2 | 2 | 2 | 1 | 2 | 6.3 | 2 | 1 | 1 | 3+3 |
| 23912694 | Kummar et al. | 50 | 31 | 31 | 0 | 100 | 0 | 11 | 0 | 1 | 1 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 23892959 | Seto et al. | | 20 | 20 | 30 | 70 | 2 | 3 | 3.5 | 2 | 2 | 1 | 2 | 10 | 2 | 2 | 1 | 3+3 |
| 23797179 | Coombes et al. | 41 | 35 | 50 | 32 | 68 | 0 | 4 | 40 | 2 | 1 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 23763921 | Reddy et al. | 29 | 25 | 25 | 72 | 28 | 2 | 4 | 13.9 | 2 | 1 | 1 | 2 | 8 | 1 | 1 | 1 | 3+3 |
| 23757357 | Sessa et al. | 48 | 101 | 101 | 68.3 | 23.8 | 8 | 8 | 35 | 1 | 1 | 1 | 1 | 7.9 | 2 | 2 | 2 | Bayesian LRM |
| 23757352 | Kordestani et al. | 90 | 28 | 28 | 57.1 | 42.9 | 3 | 5 | 7 | 2 | 1 | 1 | 1 | 10.7 | 2 | 2 | 1 | 3+3 |
| 23741066 | Venugopal et al. | 48 | 73 | 92 | 30.4 | 69.6 | 10 | 13 | 6 | 1 | 1 | 3 | 2 | 10.9 | 2 | 1 | 1 | Accelerated titration followed by 3+3 |
| 23665866 | Turkington et al. | | 19 | 19 | 47.4 | 47.4 | 7 | 3 | 1.9 | 2 | 2 | 1 | 1 | 36.8 | 1 | 1 | 1 | 3+3 |
| 23647373 | Ossenkoppele et al. | 31 | 18 | 18 | 100 | 0 | 0 | 5 | 32 | 1 | 1 | 1 | 2 | 0 | 2 | 2 | 1 | 3+3 |
| 23645447 | Richter et al. | 41 | 18 | 18 | 16.7 | 83.3 | 5 | 3 | 1.5 | 2 | 2 | 1 | 2 | 27.8 | 2 | 2 | 1 | 3+3 |
| 23589215 | Tjulandin et al. | 16 | 28 | 28 | 67.9 | 32.1 | 3 | 4 | 6 | 1 | 1 | 1 | 2 | 10.7 | 2 | 2 | 1 | 3+3 |
| 23575478 | Jimeno et al. | 43 | 44 | 94 | 64.9 | 35.1 | 7 | 9 | 8 | 1 | 1 | 1 | 2 | 7.4 | 2 | 1 | 1 | Accelerated titration followed by 3+3 |
| 23530663 | Goldman et al. | 44 | 47 | 53 | 66 | 34 | 4 | 11 | 28.6 | 1 | 1 | 1 | 1 | 7.5 | 2 | 2 | 1 | 3+3 |
| 23519998 | Kelley et al. | 41 | 16 | 25 | 0 | 100 | 4 | 2 | 1 | 2 | 2 | 1 | 3 | 16 | 2 | 1 | 1 | 3+3 |
| 23515411 | Holkova et al. | 45 | 28 | 28 | 50 | 50 | 2 | 6 | 3 | 2 | 2 | 2 | 3 | 7.1 | 1 | 1 | 1 | 3+3 |
| 23413279 | Yamamoto et al. | | 47 | 47 | 87.2 | 12.8 | 3 | 7 | 5.1 | 2 | 1 | 2 | 2 | 6.4 | 1 | 1 | 1 | 3+3 |
| 23403629 | Gojo et al. | 66 | 21 | 21 | 0 | 100 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 9.5 | 2 | 2 | 1 | 3+3 |
| 23385375 | DeAngelo et al. | 41 | 135 | 135 | 30.7 | 60 | 28 | 12 | 2 | 2 | 1 | 4 | 2 | 20.7 | 2 | 2 | 2 | Bayesian LRM with overdose control |
| 23377661 | Sharma et al. | | 30 | 30 | 46.7 | 53.3 | 16 | 8 | 6 | 2 | 1 | 2 | 1 | 53.3 | 2 | 1 | 1 | Accelerated titration followed by 3+3 |
| 23377661 | Sharma et al. | | 33 | 56 | 28.6 | 30.4 | 16 | 4 | 2 | 2 | 1 | 2 | 1 | 28.6 | 2 | 2 | 2 | Bayesian LRM with overdose control |
| 23321953 | Park et al. | 58 | 28 | 28 | 75 | 25 | 2 | 6 | 7 | 2 | 1 | 1 | 2 | 7.1 | 2 | 2 | 1 | 3+3 |
| 23299791 | Delord et al. | | 21 | 27 | 22.2 | 77.8 | 9 | 4 | 3 | 2 | 1 | 1 | 2 | 33.3 | 2 | 2 | 1 | 3+3 |
| 23256542 | Walker et al. | 36 | 11 | 11 | 0 | 100 | 4 | 1 | 1 | 2 | 2 | 1 | 1 | 36.4 | 2 | 2 | 1 | 3+3 |
| 23228990 | Raez et al. | 53 | 34 | 34 | 47.1 | 52.9 | 0 | 8 | 31.5 | 2 | 2 | 3 | 1 | 0 | 2 | 2 | 1 | Accelerated titration followed by 3+3 |
| 23211938 | Mita et al. | 43 | 147 | 147 | 27.9 | 59.9 | 15 | 10 | 2 | 2 | 1 | 7 | 2 | 10.2 | 2 | 1 | 1 | 3+3 |
| 23170896 | Anthoney et al. | | 26 | 32 | 46.9 | 50 | 5 | 6 | 10 | 1 | 1 | 1 | 1 | 15.6 | 2 | 2 | 1 | 3+3 |
| 23058787 | Gallerani et al. | 28 | 26 | 38 | 44.7 | 55.3 | 9 | 7 | 15 | 1 | 1 | 1 | 1 | 23.7 | 2 | 2 | 1 | 3+3 |
| 23054206 | Gordon et al. | 24 | 28 | 28 | 39.3 | 60.7 | 5 | 3 | 4 | 1 | 2 | 1 | 2 | 17.9 | 2 | 2 | 1 | 3+3 |
| 22935583 | Naing et al. | 28 | 49 | 49 | 57.1 | 42.9 | 8 | 5 | 9 | 1 | 1 | 2 | 2 | 16.3 | 2 | 2 | 1 | 3+3 |
| 22915658 | Yeo et al. | | 18 | 18 | 66.7 | 33.3 | 4 | 3 | 2.3 | 2 | 1 | 1 | 1 | 22.2 | 2 | 2 | 1 | 3+3 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22847785 | Mortazavi et al. | 76 | 30 | 36 | 16.7 | 83.3 | 16 | 5 | 0.8 | 2 | 2 | 2 | 1 | 44.4 | 2 | 1 | 1 | 3+3 |
| 22843927 | Schwarzlose et al. | 21 | 15 | 15 | 60 | 40 | 0 | 3 | 4 | 2 | 2 | 1 | 2 | 0 | 2 | 1 | 1 | 3+3 |
| 22843211 | Asahina et al. | 16 | 19 | 19 | 52.6 | 36.8 | 1 | 3 | 9 | 2 | 1 | 1 | 2 | 5.3 | 2 | 2 | 1 | 3+3 |
| 22761467 | Martinez et al. | 34 | 52 | 52 | 25 | 69.2 | 10 | 7 | 27 | 1 | 1 | 3 | 2 | 19.2 | 1 | 1 | 1 | Accelerated titration followed by 3+3 |
| 22735906 | Doi et al. | | 21 | 21 | 71.4 | 28.6 | 4 | 4 | 2.3 | 2 | 1 | 1 | 2 | 19 | 2 | 2 | 1 | 3+3 |
| 22711542 | Baker et al. | | 10 | 10 | 100 | 80 | 1 | 5 | 1.5 | 2 | 1 | 2 | 1 | 10 | 2 | 2 | 1 | 3+3 |
| 22644799 | Fury et al. | 60 | 18 | 18 | 66.7 | 33.3 | 1 | 3 | 1.7 | 2 | 2 | 1 | 2 | 5.6 | 1 | 1 | 1 | 3+3 |
| 22615058 | Gerecitano et al. | | 25 | 29 | 31 | 31 | 2 | 4 | 5.5 | 1 | 1 | 1 | 2 | 6.9 | 2 | 2 | 1 | 3+3 |
| 22547604 | Krop et al. | 76 | 64 | 103 | 58.3 | 41.7 | 17 | 9 | 7.1 | 2 | 1 | 3 | 2 | 16.5 | 1 | 1 | 1 | Accelerated titration followed by 3+3 |
| 22536933 | Jones et al. | | 36 | 36 | 27.8 | 72.2 | 6 | 4 | 1.2 | 2 | 2 | 2 | 1 | 16.7 | 1 | 1 | 1 | 3+3 |
| 22529266 | Tolcher et al. | 29 | 105 | 110 | 0 | 0 | 4 | 9 | 90 | 1 | 1 | 3 | 2 | 3.6 | 1 | 1 | 1 | 3+3 |
| 22382883 | Calvo et al. | | 29 | 36 | 63.9 | 52.8 | 7 | 5 | 1.8 | 2 | 1 | 1 | 2 | 19.4 | 2 | 1 | 1 | 3+3 |
| 22362161 | Dong et al. | | 31 | 31 | 71 | 29 | 2 | 5 | 6.5 | 1 | 1 | 2 | 2 | 6.5 | 1 | 1 | 1 | 3+3 |
| 22345119 | Doebele et al. | 23 | 19 | 26 | 0 | 0 | 17 | 3 | 4 | 2 | 2 | 1 | 2 | 65.4 | 2 | 2 | 1 | 3+3 |
| 22234637 | Toshihiko et al. | | 12 | 16 | 0 | 100 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 12.5 | 2 | 2 | 1 | 3+3 |
| 22139909 | Khoury et al. | 28 | 36 | 36 | 36.1 | 41.7 | 8 | 6 | 1.8 | 2 | 1 | 2 | 1 | 22.2 | 2 | 2 | 1 | 3+3 |
| 21964801 | Morita et al. | 16 | 14 | 14 | 42.9 | 57.1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 7.1 | 2 | 2 | 1 | 3+3 |
| 21494838 | Kantarjian et al. | | 30 | 47 | 40.4 | 23.4 | 4 | 6 | 8 | 2 | 1 | 1 | 1 | 8.5 | 2 | 2 | 1 | 3+3 |
| 21424701 | Holen et al. | | 21 | 21 | 33.3 | 66.7 | 4 | 4 | 2.8 | 1 | 1 | 1 | 1 | 19 | 2 | 1 | 1 | 3+3 |
| 21225315 | Tevaarwerk et al. | 16 | 24 | 24 | 66.7 | 20.8 | 1 | 6 | 2.7 | 2 | 1 | 1 | 2 | 4.2 | 2 | 2 | 2 | TITE-CRM |
| 23040438 | Weber et al. | 51 | 30 | 34 | 82.4 | 17.6 | 3 | 5 | 2 | 2 | 2 | 1 | 3 | 8.8 | 2 | 1 | 1 | 3+3 |

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## Chapter 1.2

**Anti-drug antibody formation in oncology: clinical relevance and challenges**

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**ABSTRACT**

In oncology, an increasing number of targeted anticancer agents and immunotherapies are of biological origin. These biological drugs may trigger immune responses which lead to the formation of antidrug antibodies (ADAs). ADAs are directed against immunogenic parts of the drug and may affect efficacy and safety. In other medical fields, such as rheumatology and hematology, the relevance of ADA formation is well established. However, the relevance of ADAs in oncology is just starting to be recognized and literature on this topic is scarce.

In an attempt to fill this gap in the literature, we provide an up-to-date status of ADA formation in oncology. In this focused review, data on ADAs was extracted from 81 clinical trials with biological anticancer agents. We found that most biological anticancer drugs in these trials are immunogenic and induce ADAs (63%). However, it is difficult to establish the clinical relevance of these ADAs. In order to determine this relevance, the possible effects of ADAs on pharmacokinetics, efficacy and safety parameters need to be investigated. Our data show that this was only done in fewer than 50% of the trials. In addition, we describe the incidence and consequences of ADAs for registered agents. We highlight the challenges in ADA detection and argue for the importance of validating, standardizing and describing well the used assays.

Finally, we discuss prevention strategies such as immunosuppression and regimen adaptations. We encourage the launch of clinical trials that explore these strategies in oncology.

## INTRODUCTION

Drug-induced immunogenicity has been recognized as a major challenge in the development of biological drugs. These biological drugs, such as proteins, peptides and antibodies, consist of large and complex structures and some of these structures may not belong to the patients' self-repertoire. Drug administration to patients may induce humoral immune responses causing the formation of antidrug antibodies (ADAs). ADAs may inactivate the drug, cause loss of targeting and/or an increased clearance of ADA-drug complexes which may lead to suboptimal exposure and loss of efficacy[1,2]. Patients who develop ADAs are also at risk for increased toxicity caused by the immune response that accompanies ADA formation, loss of drug targeting or formation of highly immunogenic complexes[3–5].

Extensive research is being conducted to study the immunogenicity of biological drugs such as anti-tumor necrosis factor α (anti-TNF-α) and factor VIII. This research is an important contribution to the current knowledge of risk factors for immunogenicity, formation and detection of ADAs, and possible strategies to prevent ADA formation. It has become clear that immunogenicity is not solely dependent on the biological drug. Emerging data indicate that the development of an immune response may be influenced by a variety of factors such as dose, administration regimen, administration route, product quality and handling, co-medication, patients' immune-status and genetic factors such as major histocompatibility genotype[2,6]. As a result, formation of ADAs is subject to a high inter-individual variability.

Although different medical fields have shown that ADA formation may have important consequences for therapy[5], little attention has been paid to ADA formation during anticancer therapy. Importantly, the risks and consequences of ADAs in oncology may not be identical to those in other fields e.g. rheumatology and haematology. There are several factors that need to be specifically considered in oncology, such as the use of immunostimulatory compounds, the substantial number of immunocompromised patients, concomitant treatment and immunosuppressing therapies.

This paper reviews the current knowledge on ADA formation in oncology, with the purpose of raising awareness and allowing a better understanding of the potential effects of ADAs. Topics that will be discussed include the incidence and clinical consequences of ADAs, the analytical methods that are used for detection, and the challenges in interpreting these data. Finally, in the last section of this review, we discuss challenges and potential strategies to deal with ADA formation in clinical practice, such as changes in the treatment regimen, and concomitant treatment with immunosuppressive drugs.

## INCIDENCE OF ADAS IN ONCOLOGY

The U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) published guidelines to recommend evaluation of immunogenicity of therapeutic proteins at the earliest stage of drug development and every subsequent stage[4,7]. Clinical evaluation is of high importance, since currently no tools are available to adequately predict clinical immunogenicity based on (pre-)clinical data. To study the reported immunogenicity of biological anticancer agents in clinical development, a focused PubMed literature review was performed including the keywords 'oncology' OR 'cancer' AND 'immunogenicity OR anti-drug-antibodies' AND 'clinical trial' NOT vaccine (full description of methods in Supplementary Material). Among the 81 reviewed studies with biological anticancer agents, ADAs were detected in 63%. This number indicates that the majority of compounds in oncology is immunogenic and induces ADA formation. Over the last years, the intrinsic immunogenicity of monoclonal antibodies (mAbs) has been reduced by the transition from murine to chimeric, humanized and fully human mAbs[8]. Our data support this as well for the mAbs used in oncology. The incidence of ADA formation was significantly less for human agents compared to humanized ($p$=0.03), chimeric ($p$=0.007) and murine agents ($p$=0.004) (figure 1).

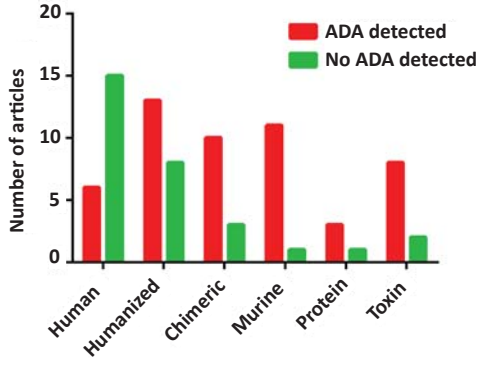


**Figure 1.** Detection of ADAs for murine, chimeric, humanized, human monoclonal antibodies, protein drugs and toxins. *Abbreviations: ADA, anti-drug antibody.*

However, even for human mAbs, ADAs are detected for 26.3%. Eight studies reported the presence of pre-existing ADAs before the start of treatment[9–15]. Although the incidence of ADAs after treatment was not significantly different between trials with and without pre-existing ADAs (75% vs. 62%, $p$= 0.70) patients with pre-existing ADAs may develop ADAs faster and in higher quantities[12]. However, these ADAs can also be transient and post-dose ADA status can become negative[10,16].

## CLINICAL RELEVANCE

In order to understand the clinical consequences of ADA formation, it is necessary to determine the impact on pharmacokinetics (PK), efficacy and toxicity. For the majority of agents, the clinical relevance of ADA formation is not well established. In clinical trial reports, the titers and percentages of ADA positive patients are often summarized, but the consequences of ADAs are not investigated. In the following sections, we discuss the relation between ADAs and these clinical parameters.

### Consequences of antidrug antibody formation on pharmacokinetics, efficacy, and toxicity

*Pharmacokinetics*

ADAs can alter the PK profile of biologicals by causing accelerated clearance of ADA-drug complexes. This can lead to a lower and even subtherapeutic exposure (area-under-the-curve (AUC)), as well as lower maximum concentrations ($C_{max}$), and a shorter elimination half-life ($T_{½}$), which have important consequences for treatment efficacy[9,14,17–22]. The impact of ADAs on PK is dependent on the affinity, the type of ADAs and the amount of free drug that is not bound to ADAs. To understand the relevance, comparing maximum concentration levels ($C_{max}$) and exposure (AUC) in both presence and absence of ADAs is essential. In the reviewed trials, data on ADAs are not routinely reported in context with PK. Among the 51 trials in which ADAs were detected, effects on PK were not explored in 67% and nine trials (18%) reported no influence of ADA formation on PK (figure 2). Only eight trials (16%) confirmed that PK was affected by ADAs. One of these, Posey *et al*., compared PK for cycle 1 and cycle 4 knowing that 50% of the patients had ADA titers[17]. All but two patients showed similar $C_{max}$ values for both cycles. One of these patients showed a very high ADA titer (460 ng/ml) and a 28% decrease in $C_{max}$. The other patient, who received a higher dose, showed a much lower ADA titer (86 ng/ml), but surprisingly showed an undetectable $C_{max}$ during cycle 4. Possibly, more high-affinity ADAs were present in this patient. This illustrates that the relationship between ADA and PK is difficult to describe and is dependent on ADA titers and affinity. Reduced drug-levels or exposures may indeed be direct results of ADA-drug binding, but may also be a consequence of increased clearance or an increase in target-mediated drug disposition. In clinical development, the use of a PK-pharmacodynamic model can provide information on the relative contribution of ADAs[23].

*Efficacy*

Even though ADAs can alter PK, this does not always translate to impaired therapeutic activity. Patients are specifically at risk of reduced efficacy if high titers of high-affinity neutralizing ADAs are present during treatment. Neutralizing ADAs bind to the variable regions of the antibody to prevent targeting, thus hampering the therapeutic activity[20]. In contrast, binding ADAs that bind to non-selective epitopes of the antibody, such as the Fc region, do not necessarily cause decreased therapeutic activity. However, both types of ADAs may lead to rapid clearance. In Yu *et al.*, neutralizing ADAs against the chimeric mAb ch14.19 were formed, which prevented binding of ch14.19 to its target disialoganglioside (GD2)[24]. Three out of eight patients in the study showed high ADA titers, yet these patients still had partial responses. Despite high titers, these ADAs may have had low affinities, or the neutralizing ADAs were formed after treatment was completed. In our dataset, out of 51 trials that detected ADA formation, 14 articles (27%) associated this with pharmacodynamic alterations or reduced efficacy, while in the majority of trials (51%) the effects were not explored (figure 2). Eleven trials (21%) found that ADAs had no effect on efficacy.

*Toxicity*

The most common toxic effects of ADAs are infusion-related reactions (IRRs)[25]. Multiple mechanisms can underly an IRR. Hypersensitivity reactions are IgE mediated[26], but IRRs can also be mediated by IgG or IgM ADAs. In hypersensitivity reactions, high titers of IgE ADAs are formed after drug exposure and bind to the FcεRI on mast cells. Upon re-exposure, drug that binds to cell-bound IgE triggers degranulation of histamine which causes an allergic reaction. As a consequence, treatment may be aborted to prevent severe allergic reactions upon retreatment[27]. In IgG-mediated reactions, binding of IgG to the drug may activate antibody-dependent cell-mediated toxicity. The Fc region of IgG ADAs binds to natural killer cells, causing a release of pro-inflammatory cytokines[28]. Furthermore, IgG aggregates and IgM are also capable of causing an inflammatory response through activation of the complement system[29]. Clinical manifestations of IRRs occur during or shortly after infusion

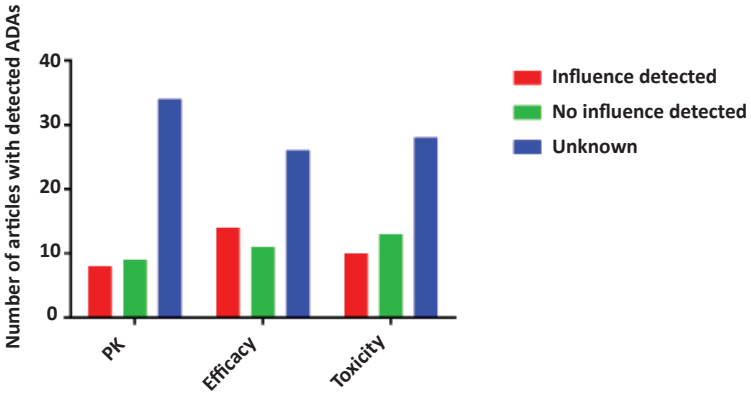


**Figure 2.** Influence of ADA formation on pharmacokinetics, efficacy and toxicity. *Abbreviations: ADAs, anti-drug antibodies; PK, pharmacokinetics.*

of the drug and include a broad range of symptoms including fever, skin rash, hypotension, gastrointestinal symptoms and more. Since clinical symptoms are similar for each mechanism it is difficult to distinguish between different types of IRR. However, IRRs may also be independent of ADA formation and vice versa[27]. An example of a non-ADA dependent IRR is cytokine release syndrome, in which cytokine-producing T-cells cause a systemic inflammatory response[26].

In the majority of studies in our dataset, the relationship between ADAs and toxicity was not investigated. For 20% of the studies, ADAs were related to IRRs such as rigors, coughing, dyspnea, back pain, rash, chills, chest tightness, hypotension, urticaria, bone pain and fever (figure 2). Besides inducing immune-mediated reactions, ADAs can also indirectly affect toxicity by causing a loss of targeting. If ADAs neutralize the therapeutic agent and prevent binding of the drug to its target, drug-induced toxicity may be decreased[30]. We hypothesize that for immunotoxins and bispecific (e.g. T-cell activating) antibodies, the effect of neutralization by ADAs may be complicated: these antibodies consist of a targeting moiety and a pharmacologically active moiety. If the ADAs neutralize the targeting moiety, the drug may cause systemic toxicity due to loss of targeting.

## CLINICAL RELEVANCE OF ADA FORMATION FOR MARKETED DRUGS

Among drugs investigated in the 81 reviewed trials, nine are currently marketed. To assess the relevance of ADAs for the agents used in clinical practice more thoroughly, we reviewed 26 EMA and FDA drug reports[31–56]. Registered drugs have overcome many obstacles in order to be approved, including the hurdle of immunogenicity. For most registered biological anticancer drugs, only a low percentage of patients form ADAs, and these ADAs often do not have a clinical effect. This is true for commonly used drugs such as cetuximab (3.4%), trastuzumab (8%), rituximab (1%–2%) and panitumumab (3.8%). Remarkably, for bevacizumab, ramucirumab, trastuzumab-emtansine, elotuzumab and blinatumomab, the clinical consequences of ADAs are unknown, despite relevant percentages of ADA positive patients (table 1). The immune checkpoint inhibitors such as nivolumab, pembrolizumab, and ipilimumab have low immunogenicity (10%, 0.4% and <2% respectively) and ADAs are thought to have little impact on efficacy. Interestingly, the percentage of patients forming ADAs against nivolumab was higher when treated in combination with ipilimumab (21.9% vs. 10% in monotherapy)[57].

For ipilimumab (monotherapy), an ADA incidence of <2% was reported. However, the assay was sensitive to drug interference, leading to a potential underestimation of the number of ADA positive patients[58]. Additional subset analyses indeed confirmed that the percentage of ADA positive patients may approach 7% instead. This demonstrates the importance of knowing the strengths and weaknesses of the assay in order to interpret the results correctly.

Tositumomab, catumaxomab, brentuximab-vedotin and aldesleukin are registered drugs which are highly immunogenic. These drugs either consist of a toxin conjugate, are a recombinant form of human protein, or are murine mAbs, and induce ADAs in 35% (brentuximab-vedotin) to 94% (catumaxomab) of patients. ADAs during tositumomab and brentuximab-vedotin therapies increase toxicity, whereas for aldesleukin only PK is affected. In all these cases, the relation to efficacy was not investigated. For catumaxomab, no clinical consequences were described in the drug report. Phase I data suggest that ADAs were formed mostly after the last infusion of catumaxomab, making it unlikely that these ADAs are clinically relevant[59].

**Table 1.** Overview of relevance of anti-drug antibodies against registered biological anticancer agents based on European Public Assessment Reports (EPARs) unless otherwise indicated[31–56]. *Abbreviations: C, chimeric; CTLA4, cytotoxic T-lymphocyte antigen 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell-adhesionmolecule; CD, cluster of differentiation; FDA, Food and Drug Administration; GD2, disialoganglioside; H, human; HER2, human epidermal growth receptor 2; HZ, humanized; I, inhibits immune system; IFN-a, interferon a; IL-6, interleukin 6; N, neutral to immune system; NA, not applicable; ND, no data; P, protein; PD-1, programmed death 1; S, stimulates immune system; SLAMF7, signaling lymphocytic activation molecule family member 7; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.*

| TYPE | DRUG | TARGET | IMMUNOSTIMULATORY EFFECT | ADAs DETECTED | FREQUENCY (%) | EFFECTS ON PK | EFFECTS ON TOXICITY | EFFECTS ON EFFICACY |
|---|---|---|---|---|---|---|---|---|
| H | Panitumumab | EGFR | N | yes | 3.8 | no | no | no |
| H | Ipilimumab | CTLA4 | S | yes | <2 | nd | nd | no |
| H | Nivolumab | PD-1 | S | yes | 10 | no | no | no |
| H | Ofatumumab | CD20 | I | no | 0 | na | na | na |
| H | Necitumumab | EGFR | N | yes | 4.1 (FDA) | nd | no | nd |
| H | Daratumumab | CD38 | I | No | 0 (FDA) | na | na | na |
| HZ | Obinutuzumab | CD20 | I | yes | 6 | nd | no | no |
| HZ | Bevacizumab | VEGF | N | yes | 0.63 (FDA) | nd | nd | nd |
| HZ | Trastuzumab | HER2 | N | yes | 8 | no | no | no |
| HZ | Ramucirumab | VEGFR2 | N | yes | 2.2 | nd | nd | nd |
| HZ | Pertuzumab | HER2 | N | yes | 3 | nd | yes | nd |
| HZ | Pembrolizumab | PD-1 | S | yes | 0.4 | no | no | no |
| HZ | Elotuzumab | SLAMF7 | S | yes | 19 (FDA) | nd | nd | nd |
| C | Rituximab | CD20 | I | yes | 1 (iv)<br>2 (sc) | no | no | no |
| C | Siltuximab | IL-6 | I | yes | 0.2 | nd | no | no |
| C | Dinutuximab | GD2 | N | yes | 17 | yes | no | nd |
| C | Cetuximab | EGFR | N | yes | 3.4 | no | no | no |
| M | Ibritumomab | CD20 | I | yes | 1.3 (FDA) | nd | no | nd |
| M | Catumaxomab | EpCAM + CD3 | S | yes | 94 | nd | no | nd |
| M | Tositumomab | CD20 | I | yes | 80 (FDA). | nd | yes | nd |
| T | Brentuximab Vedotin | CD30 | I | yes | 35 | no | yes | no |
| T | Trastuzumab -emtansine | HER2 | N | yes | 5.3 | nd | nd | nd |
| P | IFNα | IFNα-R | S | yes | 2.9 | nd | no | no |
| P/H | Aflibercept | VEGF | N | yes | 3.8 | no | no | no |
| P | Aldesleukin | IL2-R | S | yes | 71 (FDA) | yes | nd | nd |
| H | Blinatumomab | CD19,CD3 | S | yes | 1.4 | nd | nd | nd |

## ASSESSMENT OF IMMUNOGENICITY

The clinical relevance can only be assessed when reliable and valid data on ADA formation are collected for the drug of interest. Whereas drug detection assays are relatively easy to develop and interpret because the detection target is clear, this is more difficult for ADA assays because the ADA population is heterogeneous. Furthermore, it is unclear which ADAs are clinically relevant and detection is complicated by interference of the drug and ADA-drug complexes. In our dataset, the most popular method for ADA detection is Enzyme Linked Immunosorbent Assay (ELISA), including direct[60], sandwich[61], bridging[62], and competitive ELISAs[16]. Other methods include high-performance liquid chromatography (HPLC)[63], electrochemiluminescence assays (ECL)[10,64–66], radiometric assays[17,67], radioimmunoassays (RIA)[63,68–70] and cytotoxicity assays[71,72] (figure 3). The results are qualitative reports of the patient's ADA status (positive/negative), often accompanied by titer levels.

For a proper understanding of assay results, it is essential to know which type of ADA is detected by the assay. ADAs may consist of multiple immunoglobulin subclasses, and are either freely circulating or drug-bound. However, most assays, including ELISAs, measure only free IgG subclasses. Drug-bound ADAs and important immunoglobulin subclasses, such as IgE, are not detected which may lead to an underestimation of the incidence and the titer of ADAs[73]. To manage drug interference, samples can be acidified in order to separate drug-ADA complexes[74]. Samples can also be taken prior to dosing, when drug concentrations are low[74]. Another option is using the antigen binding test (ABT). ABTs are less vulnerable to drug interference and can measure moderate amounts of ADA-drug immunocomplexes[5]. In this assay, ADAs of the IgG class, including those that are drug-bound, are pulled down during the first step of the assay using protein A. Then, radiolabeled drug binds to the ADA, and the radiation signal is measured (figure 3). If the samples are acidified prior to the ABTs, the assay is even more tolerant to drug interference[5]. However, in spite of their increased resistance to drug interference, even ABTs may give an underestimation, as not all immunoglobulin subtypes are measured.

Different assays detect different subclasses and idiotypes of ADAs, and currently no assay is able to detect all ADAs. This is one of the reasons why ADA formation across different trials cannot be accurately compared. To increase sensitivity, a tiered approach can be applied, consisting of a screening assay, a confirmatory assay and finally characterization of the ADAs[75]. In a number of trials, ADAs were detected already prior to treatment, and these samples were occasionally deemed false-positive[15]. By using the aforementioned tiered approach, these samples should be analyzed for ADA with a confirmatory assay in order to truly validate that these patients are ADA negative. An example of this approach is the phase I trial of AGS-1C4D4, a human anti-prostate stem cell antigen monoclonal antibody[76]. An ECL test served as the screening test in which three patients were tested ADA positive. A second assay was performed for confirmation, which yielded negative results. Therefore, patients were considered negative for the presence of anti-AGS-1C4D4 antibodies.

Although accurately detecting the presence and incidence of ADAs is important, it may be even more crucial to characterize the effects of the detected ADAs. Assays that determine the presence of neutralizing antibodies, such as cytotoxicity assays[72], can select for those ADAs that affect efficacy.

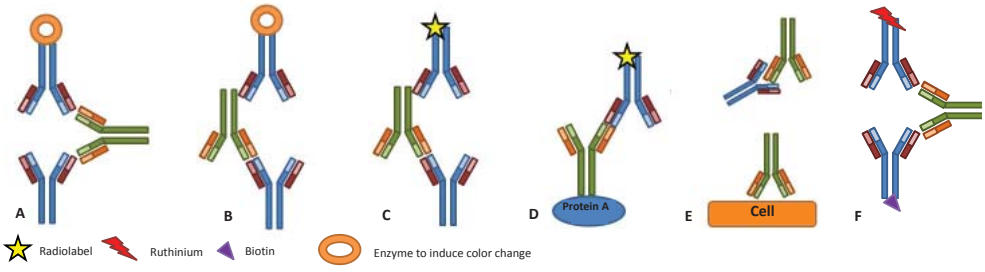


**Figure 3.** Schematic representation of techniques used to detect ADAs | **A** | Bridging ELISA with drug as binding agent and enzyme-linked drug as idiotype-detecting agent | **B** | Sandwich ELISA with drug as binding agent and enzyme-linked secondary antibody as isotype-detecting agent | **C** | RIA with radiolabeled drug binding to ADAs | **D** | ABT in which IgG from serum is pulled down by protein A bound to a solid carrier, radiolabeled drug is added and binds to ADAs | **E** | Cytotoxicity assays measure ADA-induced alterations in cytotoxic effects of the drug | **F** | Bridging electrochemiluminescence assays measure electrochemical signals from the ruthinium-labeled drug bound to the ADA-biotin-streptavidin complex.

To summarize, ADA assays should be rationally designed to detect the most relevant range of ADAs, and results should be consistently reported to allow an understanding of the characteristics and consequences of the detected ADAs. Furthermore, standardization of assays is essential to allow comparison of results on ADA formation between different trials. For this, the recently developed guidelines for ADA assays for clinical use published by the ABIRISK consortium could be used[75].

## PREVENTION STRATEGIES

Although reducing intrinsic immunogenicity of the drug is a successful approach to reduce ADA formation, clinical results show that this is not sufficient to prevent ADA formation in all patients. Several prevention strategies have been applied in clinical practice and their potential will be explored in this section.

### Tolerance induction by adapations to the treatment regimen

Several studies indicate that immunogenicity can be reduced by increasing the exposure through high-dose and high-frequency therapy[5,27,77–80]. The effects of high-dose and high-frequency treatment were first observed in haemophilia patients treated with factor VIII after the doses were increased from normal treatment regimen to twice daily infusions[80]. In patients treated with infliximab, the incidence of ADA formation was 28% after a single dose of infliximab compared to 6% after repeated doses[81,27]. It is hypothesized that the tolerance is mediated by activation of regulatory T-cells[82], and apoptosis of effector T-cells[83]. However, it is unknown if this is a consequence of increased plasma concentrations ($C_{max}$, $C_{ss}$), prolonged exposure ($T_{1/2}$), higher exposure (AUC), or any combination of these.

In oncology, the effects of modifications to the treatment regimen are conflicting. Among the nine studies that reported ADA formation for different doses, the majority found that ADA formation was not dose-dependent [21,71,84–86], and only two studies confirmed a decrease in ADA formation with higher doses[17,19].
The main limitations of high-dose or high-frequency treatment are the therapeutic and toxic effects of the drug. One possible method to avoid these, is by administering only the immunogenic part of the molecule without the pharmacologically active moiety, as was done by Somerfield *et al.*[78]. In this study, patients treated with alemtuzumab received the non-binding SM3 shortly prior to treatment. SM3 differs from alemtuzumab in only a single point mutation, which prevented binding to CD52. In this way, high doses may be administered without causing unacceptable toxicity. This strategy reduced the percentage of ADA positive patients significantly from 74% to 21%. However, introducing this additional compound into the clinic may be very costly and time-consuming, and occupation of the target by this compound may be a problem.
In contrast to the results of high-dose and high-frequency treatment, four studies report that tolerance was induced by decreasing the exposure through lower doses, continuous infusion or subcutaneous administration[71,87–89]. For the humanized antibody trastuzumab, ADA formation was twice as high after intravenous administration (14.6%) as after subcutaneous administration (7.1%) in equivalent doses[89]. For the anti-mesothelin immunotoxin SS1P, a bolus injection administered in three times every other day induced ADAs in 88%, whereas an equivalent dose of a ten-day continuous infusion induced ADAs in 75%[72].
In summary, it is clear that adapations to the dose and treatment regimen can alter immunogenicity. Most evidence is available for tolerance induction by high-dose and high frequency therapy but this appears not effective for all drugs. Modifications to the treatment regimen are relatively easy adjustments and should be considered based on successful cases that have been described in literature.

### Immunosuppression

In rheumatology, the use of immunosuppressive agents is an effective treatment strategy that simultaneously reduces the frequency of ADA formation up to 46%[90–94]. Concomitant treatment with methotrexate (MTX) in low (5-10 mg), intermediate (12.5 – 20 mg), or high weekly doses (>22.5 mg) successfully led to reduction of ADA formation in adalimumab treated rheumatoid arthritis patients in a dose-dependent manner[90]. A similar effect was observed in rheumatoid arthritis patients who received infliximab. After a single dose of infliximab, ADAs were formed in 53%, 21% and 7% (1, 3, and 10 mg infliximab/kg) of the patients. When combined with 7.5 mg MTX weekly, the incidence was respectively 15%, 7% and 0%[92]. Azathioprine, 6-mercaptopurine, hydrocortisone, and rituximab have also been applied in rheumatology, but results have been inconclusive[94–97].
In oncology, immunosuppression can be effective for the treatment of hematological malignancies, but for many solid tumors immunosuppression may be undesired. Among the articles we reviewed, only two investigated the effects of immunosuppression and showed that cyclophosphamide and cyclosporin could not prevent ADA formation[98,99]. Unique challenges regarding the use of immunosuppression to prevent ADA formation in oncology may be the large group of immunocompromised patients and the increasing use of immunostimulatory agents such as immunotoxins, interleukin-2, CD3-, CD19-, CD28 agonists, anti-programmed death 1 and anti-cytotoxic

T-lymphocyte antigen 4. Both factors may alter the risk of ADA formation (decrease and increase, respectively) and for these patients special prevention strategies may be required. Our data showed no significant difference (*p*=1.0*)* in ADA formation between the trials with immunostimulatory agents (75% detected ADAs (n=20)), immunosuppressing agents (69% (n=13)) and non-immunotherapies (56% (n=48)) (figure 4). No data were available to compare ADA formation between immunocompromised and immunocompetent patients. Although some trials investigated immunostimulatory agents combined with immunosuppression, effects on treatment efficacy and ADA formation could not be determined based on the reported data[18]. However, it is clear that despite immunosuppression, patients are still at risk of ADA formation[23,100]. This is illustrated by the trial by Welt *et al.*[30] with the humanized antibody huA33 in which concomitantly administered chemotherapy led to bone marrow suppression in ten out of 16 patients. The majority of ADA negative patients were immunocompromised (4 out of 6), but one patient with severe neutropenia showed high and increasing ADA titers.

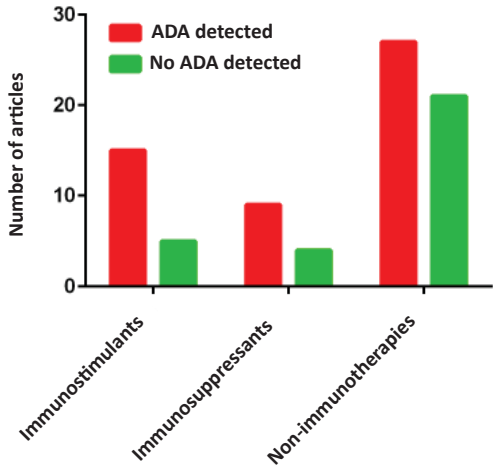


**Figure 4.** Detection of ADAs for immunostimulants, immunosuppressants and non-immunotherapies.

A feasible prevention strategy for oncology may be targeted B-cell inhibition with anti-CD20 agents such as rituximab, veltuzumab or obinutuzumab which inhibit *de novo* humoral antibody responses. Several trials have been done with B-cell inhibiting agents but these did not detect effects on ADA formation[61,69,72,87,101,102]. Hassan *et al.*[103] showed that rituximab was able to induce full depletion of CD20 positive B-cells, but this did not prevent ADAs targeted towards the therapeutic drug. Maeda *et al.*[104] described a case of a rituximab treated mantle-cell lymphoma patient, who developed high titers of anti-rituximab antibodies leading to a decreased exposure, and Sausville *et al.* [69] detected ADAs in 75% of B-cell lymphoma patients treated with the B-cell targeting anti-CD22 immunotoxin IgG-RFB4-SMPT-dgA.

These trials show that ADA formation is still possible despite B-cell depletion, but it is not clear if the frequencies, titers or onset may be reduced. Taken together, immunosuppression has successfully reduced ADA formation in rheumatology but evidence for immunosuppression in oncological patients, and in combination with immunotherapies or immunocompromisation is lacking. The absence of observed effects of immunosuppression on ADAs may be explained by the fact that these clinical trials were not designed to investigate this thoroughly. Clinical trials specifically designed to determine the effect of immunosuppressive therapy, such as anti-CD20, on anti-drug antibody formation may determine whether immunosuppression is useful in oncology.

## CONCLUSIONS

We confirmed that the majority of biological anticancer agents in clinical development induces ADA formation. For most agents that were EMA or FDA approved, ADAs have been detected but have not been an obstacle for approval. However, even among marketed agents, important gaps in the data on ADA formation exist. In most cases the consequences of ADAs for efficacy, pharmacokinetics and toxicity are not thoroughly investigated. Routine investigation of the relationship between ADAs and these parameteres may help to establish the clinical relevance and explain variability in drug responses and safety.
Furthermore, inconsistent reporting and heterogeneity in detection methods complicate interpretation of the obtained results regarding ADA formation. Consistent reporting of the method of assessment, the incidence and characteristics of the detected ADAs will allow proper interpretation and comparison of the relevance of ADA formation. We would like to encourage the use of standardized terms for immunogenicity reporting as published by the ABIRISK consortium[75].

If ADAs are considered clinically relevant for a specific agent, strategies for prevention or management of the consequences may be designed. One potential method that is quick and easy to investigate is regimen adjustment. Although the mechanisms are not yet fully understood, clinically relevant effects have been observed, as we described in this review. More aggressive measures to be considered include immunosuppressive treatment with for example anti-CD20 or methotrexate, although more research is needed to evaluate whether these methods are feasible in oncology.

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56 European Medicines Agency. Perjeta (pertuzumab) Eur. Public Assess. Rep. 2016.
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**SUPPLEMENTARY MATERIAL - DATA TABLE INCLUDED TRIALS**

| PHASE | TYPE | DRUG | TARGET | TYPE OF DRUG | IMMUNOSTIMULATORY EFFECT | ADMINISTRATION ROUTE | INFUSION TIME | ADAs DETECTED | EFFECTS ON PK | EFFECTS ON TOXICITY | EFFECTS ON EFFICACY | AUTHOR | PMID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | C | 90yttrium-labeled cT84.66 | CEA | IgG1 | N | iv | nd | yes | nd | nd | nd | Wong | 11051230 |
| 1 | C | 90yttrium-labeled cT84.66 | CEA | IgG1 | N | iv | 25 | no | na | nd | nd | Wong | 9867150 |
| 1 | C | 90Y-DOTA-cT84.66 | CEA | IgG1 | N | iv | 25 | yes | yes | yes | yes | Wong | 16706629 |
| 1 | H | MEDI-575 | PDGFR-α | IgG2k | N | iv | 60/90 | yes | no | no | no | Becerra | 25149088 |
| 1 | H | MEDI-575 | PDGFR-α | IgG2k | N | iv | 60 | no | na | nd | nd | Murakami | 26223436 |
| 1 | H | AGS-PSCA | PSCA | IgG1k | N | iv | 60/120 | no | na | nd | nd | Morris | 22553195 |
| 1 | HZ | dacetuzumab (SGN-40) | CD40 | IgG1 | S | iv | nd | no | na | nd | nd | Vos | 24919462 |
| 1 | H | MABp1 | IL-1α | IgG1k | I | iv | 60 | no | na | nd | nd | Hong | 24746841 |
| 1 | HZ | TB-403 (RO5323441) | PlGF | IgG1 | N | iv | 60 | yes | nd | nd | nd | Martinsson-Niskanen | 21906811 |
| 1 | T | B9E9FP | CD20 | IgG1 | I | iv | 15/30 | yes | nd | no | nd | Forero | 14996706 |
| 1 | H | lexatumumab (HGS-ETR2) | TRAIL-R2 | IgG1L | N | iv | 30/ 120 | no | na | nd | nd | Wakelee | 19633048 |
| 1 | HZ | AS1402 | MUC1 | IgG1k | N | iv | 60/120/180 | no | na | nd | nd | Pegram | 19811637 |
| 1 | H | MEDI-573 | IGFI and IGFII | nd | N | iv | 60/90 | no | na | nd | nd | Haluska | 25024259 |
| 1 | HZ | onartuzumab | MET | nd | N | iv | 30/60/90 | yes | no | no | nd | Salgia | 24493831 |
| 1 | H | AGS-1C4D4 | PSCA | IgGk | N | iv | 60/120 | no | na | nd | nd | Antonarakis | 22020316 |
| 1 | T | moxetumomab pasudotox (CAT-8015; HA22) | CD22 | nd | I | iv | 30 | yes | nd | nd | yes | Kreitman | 22355053 |
| 1 | T | glembatumumab vedotin | gpNMB | IgG2 | N | iv | 90 | yes | no | nd | nd | Ott | 25267741 |
| 1 | HZ | 90Y-clivatuzumab traxetan (90Y hPAM4) and 111In-hPAM4 | pancreatic cancer mucin glycoprotein | nd | N | iv | 10 | yes | nd | no | nd | Gulec | 21527562 |
| 1 | HZ | bivatuzumab (BIWA 4) | CD44v6 | IgG1 | I | iv | nd | yes | nd | nd | nd | Borjesson | 14506195 |
| 1 | HZ | sibrotuzumab | FAP | IgG1 | N | iv | 60 | yes | yes | yes | yes | Scott | 12738716 |
| 1 | C | IMC-1C11 | KDR | IgG1 | N | iv | 60 | yes | yes | nd | nd | Posey | 12684400 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | I131-M195 | CD-33 | IgG2a | I | iv | 20 | yes | yes | yes | yes | Caron | 8306247 |
| 1 | M | OKB7-I131 | CR-2 on B cells | IgG2b | I | iv | 60 | yes | nd | nd | nd | Scheinberg | 2332769 |
| 1 | HZ | huA33 | cell surface differentiation antigen Mr 43,000 | IgG1 | N | iv | 1mg/min/m2 | yes | nd | yes | nd | Welt | 12684403 |
| 1 | M | MikB1 | CD122 | IgG2a | I | iv | nd | no | na | na | na | Morris | 16387851 |
| 1 | HZ | 111In-DTPA- EGFR | EGFR | na | N | iv | nd | no | na | na | na | Vallis | 24753984 |
| 1 | C | Br96- DOX | Lewis-Y | IgG1 | N | iv | 2-24 hours | yes | no | nd | nd | Saleh | 10829049 |
| 1 | C | ch18.17 | gd2 | IgG3, IgG1k | N | iv | 60 | yes | nd | nd | yes | Yu | 9626218 |
| 1 | C | mCC49 | TAG-72 | IgG4 | S | iv | 30 | yes | nd | no | nd | Forero | 16248762 |
| 1 | C | mCC49 | TAG-72 | IgG4 | S | iv | 30 | yes | nd | no | nd | Forero | 16248762 |
| 1 | HZ | bivatuzumab-mertansine | CD44v6 | IgG1 | S | iv | 30 | no | na | na | na | Sauter | 17332932 |
| 1 | C | T84.66 | CEA | IgG1 | N | iv | 25 | yes | nd | nd | nd | Wong | 7493373 |
| 1 | HZ | 212Pb-trastuzumab | HER2 | IgG1 | N | iv and ip | nd | no | na | na | na | Meredith | 25157044 |
| 1 | M | HRS-3/A9 | CD16 and CD30 | IgG | S | iv | 60 | yes | nd | yes | yes | Hartmann | 9869203 |
| 1 | T | SGN10 | Lewis-Y | na | S | iv | 5 | yes | yes | no | nd | Posey | 12374676 |
| 1 | P | rHuIL-12 | IL-12r | na | S | sc | bolus | no | na | na | na | Gokhale | 24725395 |
| 1 | H | ipilimumab | CTLA4 | IgG1 | S | iv | nd | no | na | na | na | Horinouchi | 25924991 |
| 1 | H | ramucirumab | VEGFR2 | IgG1 | N | iv | nd | yes | nd | nd | nd | Chiorean | 25787923 |
| 2 | HZ | tigatuzumab | DR5 | IgG1 | N | iv | nd | no | na | na | na | Forero-Torres | 25779953 |
| 1 | HZ | TAS266 | DR5 | nd | N | iv | 60 | yes | nd | yes | yes | Papadopoulos | 25721064 |
| 1 | C | siltuximab | IL-6 | IgG1k | I | iv | 60 | no | na | na | na | Suzuki | 25655379 |
| 1 | H | PATSM6 | GRP78 | IgM | N | iv | 90 | no | na | na | na | Rasche | 25637055 |
| 3 | HZ | trastuzumab | HER2 | IgG1 | N | iv | nd | yes | no | no | no | Jackisch | 25403587 |
| | | | HER2 | | | sc | | yes | | | | | |
| 1,2 | M | catumaxomab | EpCAM + CD3 | IgG2a, IgG2b | S | ip | 180 | yes | nd | no | no | Pietzner | 25367854 |
| 1 | HZ | epratuzumab tetratexan radiolabeled | CD22 | nd | I | iv | veltuzumab 60 m tetratexan epratuzumab few min | yes | nd | nd | nd | Witzig | 25150258 |
| 2 | H | mapatumumab | TRAIL-R1 | nd | N | iv | 60 | yes | nd | nd | nd | Pawel | 24560012 |
| 2 | H | aflibercept | VEGF | IgG1 | N | iv | 60 | yes | nd | no | nd | Tew | 24127346 |
| 2 | H | carlumab | CCL2 | IgGk | N | iv | 90 | no | na | na | na | Pienta | 22907596 |
| 1 | H | ct-322 | VEGF-R2 | nd | N | iv | 60 | yes | no | nd | no | Tolcher | 21224368 |
| 2 | M | catumaxomab | EpCAM + CD3 | IgG2a, IgG2b | S | ip | 360 | yes | no | no | no | Ruf | 20565453 |
| 1 | H | IMC-11F8 | EGFR | IgG1 | N | iv | nd | no | na | na | na | Kuenen | 20197484 |
| 1 | HZ | tigatuzumab | DR-5 | IgG1 | N | iv | 90/60/30 | no | na | na | na | Forero-Torres | 20187792 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | T | VB845 | EpCAM | na | S | it | bolus | yes | yes | yes | yes | MacDonald | 19920898 |
| 1 | P | hu14.18-IL2 | IL-2r | na | S | iv | 240 | yes | yes | nd | no | Hank | 19737959 |
| 1 | T | SS1P | Mesothelin | na | S | iv | 10 days | yes | nd | nd | yes | Kreitman | 19671873 |
| 1, 2 | HZ | veltuzumab/huA20 | CD20 | nd | I | iv | 1-6 hours | yes | no | no | no | Morschhauser | 19451441 |
| 1 | T | VB845 | EpCAM | na | S | it | bolus | yes | yes | nd | no | MacDonald | 19016010 |
| 1 | T | MLN2704 | PSMA expressing cells | na | S | iv | 150 | no | na | na | na | Galsky | 18362364 |
| 1 | H | panitumumab | EGFR | IgG2 | N | iv | 60/120 | no | na | na | na | Weiner | 18223225 |
| 1 | H | lexatumumab (HGS-ETR2) | TRAIL-R2 | IgG1L | N | iv | 30-120 | no | na | na | na | Plummer | 17947486 |
| 1 | HZ | etaracizumab | αvβ3integrine receptor | nd | N | iv | 30 | no | na | na | na | Delbaldo | 17876527 |
| 1 | H | adecatumumab | EpCAM + CD3 | IgG1 | N | iv | 30 | no | no | na | na | Oberneder | 16930989 |
| 1, 2 | HZ | h-R3 | EGFR | IgG2a | N | iv | 60 | yes | no | no | no | Ramos | 16575203 |
| 0 | HZ | DOTA labeled J591 | PSMA | IgG1 | N | iv | 5 mg/min | no | no | na | na | Morris | 16243819 |
| 1 | HZ | huA33 | cell surface differentiation antigen Mr 43,000 | IgG1 | N | iv | nd | yes | nd | yes | nd | Ritter | 11559561 |
| | | huA33 | | | | | | | | | | | |
| | | radiolabeled huA33 131I | | | | | | | | | | | |
| 1 | T | S16020 | topisomerase II | na | N | iv | 60 | no | na | na | na | Awada | 12453862 |
| | | | | | | | 180 | | | | | | |
| | | | | | | | 60 | | | | | | |
| 1 | M | lor-egf/r3 | EGFR | IgG2a | N | iv | 120 | yes | nd | yes | yes | Crombet | 11394532 |
| 1 | M | lor-egf/r3 | EGFR | IgG2a | N | iv | 120 | yes | nd | nd | no | Crombet | 11279803 |
| 1 | M | CD3 CD19 + CD 28 | CD3 CD19 +CD28 | IgG2a, IgG1k | S | intralymphatic | bolus | yes | nd | nd | nd | Manzke | 11251974 |
| 1 | C | 99Tm-U36 | CD44v6 | IgG | N | iv | nd | yes | yes | nd | nd | Colnot | 11138685 |
| 1 | T | anti-Tac(Fv)-PE38 (LMB-2) | CD25 | na | S | iv | 30 | yes | nd | yes | yes | Kreitman | 10764422 |
| 1 | HZ | hOKT3gamma4 | CD3 | IgG4 | S | iv | 10 | yes | no | nd | nd | Richards | 10232594 |
| 1, 2 | M | 131I- G250 | G250 | nd | N | iv | bolus | yes | nd | nd | yes | Divgi | 9829736 |
| 1 | C | CLBIL6/8 | IL-6 | IgG1k | I | iv | 120 | no | na | na | na | Van Zaanen | 9722307 |
| 1, 2 | M | HRS-3/A9 | CD16/CD30 | IgG1 | S | iv | 60 | yes | nd | yes | nd | Renner | 9435869 |
| 1 | C | 111In-cT84.66 | CEA | IgG1 | N | iv | 25 | yes | nd | nd | nd | Wong | 9430476 |
| 1 | T | IgG-RFB4-SMPT-dgA | CD22 | IgG1k | I | iv | 8 days | yes | nd | nd | nd | Sausville | 7780133 |
| 1 | HZ | M195 | CD33 | IgG2a | I | iv | 20 | yes | no | nd | no | Caron | 8142644 |
| 1 | C | L6 | L6 | IgG1 | N | iv | 4-16 hours | yes | nd | nd | nd | Goodman | 8382560 |
| Pilot | P | IL2 | IL2r | na | S | il | 0,5 ml/min | yes | no | no | no | Sarna | 2319262 |
| 1 | H | 105AD7 | 791T/36 | IgG1 | N | im | bolus | no | na | na | na | Robins | 1913661 |
| 2 | P | IL2 | IL2r | na | S | sc | bolus | yes | nd | nd | no | Kirchner | 2222898 |
| | | IFN-a2B | IL2r | | | | | no | na | na | na | | |

| ADA TYPE | ANALYTIC METHOD | CO-MEDICATION | CANCER | REGIMEN | TIMEPOINTS ANALYSIS | AUTHOR | PMID |
|---|---|---|---|---|---|---|---|
| HPLC detects anti-idiotype | RIA and HPLC | DTPA | metastatic CEA-producing malignancies | first dose is 5mCI 111ln-labeled DTPA-cT84.66 (5mg). After one week, 12mCi/m2 90Y-labeled DOTA cT84.66, up 3 cycles of therapy q6w. | baseline and postdose week 2, 1, 3, 6 months | Wong | 11051230 |
| HPLC detects anti-idiotype | RIA and HPLC | none | colorectal cancer | single dose, 0 20 or 100 mg DTPA-cT84.66. After that 5 mg 111ln DTPA-cT84.66 | baseline and postdose week 2, 1, 3, 6 months | Wong | 9867150 |
| HPLC detects anti-idiotype | RIA and HPLC | none | metastatic CEA-producing malignancies | first dose is 5mCI 111ln-labeled DOTA-cT84.66 (5mg). After one week, 5 -22 mCi/m2 90Y-labeled DTPAcT84.66, up 3 cycles of therapy q6W. | baseline and postdose week 2, 1, 3, 6 months | Wong | 16706629 |
| anti-idiotype | bridging ECL | none | advanced solid tumors | 6, 9, 12, or 15 mg/kg qW; 25 and 35 mg/kg q3W<br>Q3W | nd | Becerra | 25149088 |
| anti-idiotype | bridging ECL | none | advanced solid tumors | part 1: 9,15,35 mg/kg/3weekly. Part 2: 25 mg/kg/3 weekly | nd | Murakami | 26223436 |
| anti-idiotype | bridging ELISA | none | castration-resistant prostate cancer | q3w (1-40mg/kg) | 3 weekly or 6 weekly | Morris | 22553195 |
| nd | ELISA | none | relapsed diffuse large B-cell lymphoma | qW with intrapatient dose escalation the first 4 wks. | nd | Vos | 24919462 |
| nd | sandwich ELISA | none | refractory cancers | q2w (3.75mg/kg) and q3w (0.25 mg/kg, 0.75 mg/kg, 1.25 mg/kg, and 3.75 mg/kg.) | baseline, day 1, 8, 15 and every 2 weeks | Hong | 24746841 |
| anti-idiotype | bridging luminescence ELISA | none | tested in healthy subjects | single dose | baseline, week 4 and 8 postdose | Martinsson-Niskanen | 21906811 |
| separate assays for detecting chimeric, mouse or streptavidin component. HACA sandwich ELISA detects H and L chain. | sandwich ELISA and bridging ELISA | DOTA | non-hodgkin lymphoma | single dose(160mg/m2 or 320mg/m2), followed by DOTA | nd | Forero | 14996706 |
| nd | direct binding ELISA and competitive binding ELISA | no | advanced solid tumors | 0.1, 0.3, 1, 3 and 10 mg/kg q2W | nd | Wakelee | 19633048 |
| nd | nd | no | Advanced breast cancer | qw to q3w (patient individualized PK assessment | nd | Pegram | 19811637 |
| anti-idiotype | nd | no | advanced solid tumors | 0.5, 1.5, 5, 10, or 15 mg/kg qW ; 30 or 45 mg/kg q3W | baseline and before every infusion | Haluska | 25024259 |
| CDR, engineered framework, or non-engineered framework. | bridging ECL and immunodepletion | none or bevacizumab | advanced solid tumors | q3W (bevacizumab also q3W) | before every infusion and EOT | Salgia | 24493831 |
| nd | ECL and immunodepletion assay | no | castration-resistant prostate cancer | q3W | baseline,4 times 3 weeklyand at safety follow-up visit | Antonarakis | 22020316 |

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|---|---|---|---|---|---|---|---|
| cytotoxicity assay detects neutralizing Abs. ELISA detects binding antibodies. | ELISA and cytotoxicity assay | no | hairy cell leukemia | QOD X3 | before every infusion | Kreitman | 22355053 |
| anti-idiotype | bridging ELISA | no | advanced melanoma | q3W; day 1, 8 of 21 day cycle; day 1,8 and 15 of a 21 day cycle | nd | Ott | 25267741 |
| nd | ELISA | RIT | advanced pancreatic carcinoma | single dose | baseline, 4, 8, and 12 weeks postdose | Gulec | 21527562 |
| anti-idiotype | bridging ELISA | RIT | HNSCC | single dose of 186Re-labeled BIWA 4 in radiation dose-escalation steps of 20, 30, 40, 50, and 60 mCi/m2. BIWA= 50mg | baseline, week 1 and 6 postdose | Borjesson | 14506195 |
| anti-idiotype | bridging ELISA | RIT | advanced or metastatic FAP positive cancer | qW, 5, 10, 25, or 50 mg/m2. Trace labeling with 8-10 mCi of 131I in wk 1,5 and 9 | before every infusion and 4 weeks after EOT | Scott | 12738716 |
| anti-idiotype | bridging radiometric assay | none | colorectal carcinoma | qW, 0.2 mg/kg, 0.6 mg/kg, 2.0 mg/kg , and 4.0 mg/kg | day 1, 22, 36, and 42 | Posey | 12684400 |
| murine part of MoAb | ELISA | none | acute and chronic myelod leukemia, myelodysplastic syndrome | 2-4 divided doses | 8 days, every 2-3 weeks | Caron | 8306247 |
| nd | sandwich ELISA | none | non-hodgkin lymphoma | single dose | day 7-10, monthly | Scheinberg | 2332769 |
| nd | surface Plasmon Resonance immunoassay | carmustine,vincristine,flu orouracil and streptozocin | colorectal cancer | 10 mg/m2/week | before every infusion | Welt | 12684403 |
| nd | ELISA | none | T cell large Granular Lymphocyte Leukemia | 1,5 mg/kg on day 1,4,7,10 | day 6 10 and week 4-6 postdose | Morris | 16387851 |
| IgG | sandwich ELISA | none | breast Cancer | single fixed dose | baseline, week 2, 4, 12 | Vallis | 24753984 |
| IgG, IgA and IgM | sandwich ELISA | none | breast and Colon | 3 weekly | baseline and EOT | Saleh | 10829049 |
| IgG against anti-idiotype | radiometric bridging assay | none | neuroblastoma, osteosarcoma | 2 -3 weekly | weekly, 2-weekly, monthly | Yu | 9626218 |
| anti-idiotype | radiometric bridging assay | paclitaxel + IFN | non small-cell lung carcinoma | different regimens | baseline and after infusion | Forero | 16248762 |
| anti-idiotype | radiometric bridging assay | EDTA + IFN | non small-cell lung carcinoma | | | Forero | 16248762 |
| anti-idiotype | radiometric bridging assay | DTPA+IFN | | | | | |
| anti-idiotype | bridging ELISA | none | head-neck small cell | 13 pts received second dose after 3 weeks | baseline, day 21 | Sauter | 17332932 |
| nd | RIA | none | CEA positive tumors | maximum 3 every 6 wks | baseline, postdose 2 wks, 1,3,6 months | Wong | 7493373 |

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|---|---|---|---|---|---|---|---|
| nd | nd | none | HER2 expressing tumors | single dose | nd | Meredith | 25157044 |
| nd | ELISA | none | CD30 hodgkins disease | 4 times every 3-4 days | baseline, 4-5 weeks postdose | Hartmann | 9869203 |
| IgG/IgM/IgA | sandwich ELISA and radiometric double antigen assay | dexamethasone | Le-Y positive tumors | day 1,4,8,11 every month | baseline day 1, 8, 11, 15, 22, 29 | Posey | 12374676 |
| nd | nd | none | tested in healthy subjects | single dose of 2,5,10,12,15,20 microgram | baseline, postdose day 28/ day 45 | Gokhale | 24725395 |
| nd | bridging ECL | paclitaxel + carboplatin | non small-cell lung carcinoma | 3 weekly, first 2 cycles of chemo before combination 3 and 10 mg | baseline, cycle 3 and prior to each infusion, EOT and follow up | Horinouchi | 25924991 |
| anti-idiotype | bridging RIA | none | advanced solid tumors | q2w 6, 8 ,10 mg/kg and q3W 15and 20 mg/kg | predose to every infusion, EOT, at 45d follow up | Chiorean | 25787923 |
| anti-idiotype | bridging ELISA | paclitaxel | triple negative breast cancer | d1,8,15 PAC + TIG on day 1 and 15 10 mg c1 and 5 mg after | predose, 4 and 8 weeks after TIF insuion and every 8 weeks, 34 months after EOT | Forero-Torres | 25779953 |
| IgG | sandwich ELISA | none | advanced solid tumors | escalating doses of TAS266 on day 1, 8, 15 and 22 | baseline, day 8, 15, 22 and EOT | Papadopoulos | 25721064 |
| nd | ELISA | bortezomib, dexamethasone | multiple myeloma | 5,5 and 11 mg/kg every 3 weeks with bortezomib and dexa | baseline, EOT and 1, 2, 3 months after EOT | Suzuki | 25655379 |
| nd | ECL | antihistaminics as premedication | multiple myeloma | escalating doses on day 1,3,8,10 | day 15 and EOT | Rasche | 25637055 |
| nd | nd | docetaxel, 5FU, eprirubicin, cyclophosphamide | HER2 positive breast cancer | q3w trastuzumab, after surgery 1 year of monotherapy | baseline, day 2,5,13,18 and EOT after 3,6,12,18, 24 months | Jackisch | 25403587 |
| nd | nd | | | | | | |
| nd | nd | paracetamol | malignant ascites | escalating doses day 0,3,7,10 | | Pietzner | 25367854 |
| nd | ELISA | anti-CD20- veltuzumab | non-hodgkin lymphoma | weekly antiCD20 4x and in week 3 and 4 anti CD22 | baseline, week 4 and 12 | Witzig | 25150258 |
| nd | ECL and inhibition assay | carboplatin, paclitaxel | non small-cell lung carcinoma | 6 x carbo, pac, mapatumumab 10 mg or 30 mg.kg q3w | baseline, before every other infusion | Pawel | 24560012 |
| nd | ELISA | none | ovarian cancer | 2mg/kg or 4 mg/kg q2w | every 8 weeks | Tew | 24127346 |
| nd | Immunoassay | corticosteroids allowed | prostate cancer | q2w | 4,8, 12 weeks after last dose and during IRRs | Pienta | 22907596 |
| IgG and IgM | sandwich ELISA | none | advanced solid tumors | qw or q2w in escalating doses | baseline, day 15, 22 and every infusion and 30 days follow up | Tolcher | 21224368 |
| anti-idiotype | bridging ELISA | paracetamol | malignant ascites | ascending doses 10,20,50,150ug on day 0,3,6,7,10 | baseline, day 2, 6, 7 | Ruf | 20565453 |

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|---|---|---|---|---|---|---|---|
| anti-idiotype | bridging radiometric assay | none | advanced solid tumors | qw or q2w in escalating doses | predose, every 6 weeks | Kuenen | 20197484 |
| anti-idiotype | bridging ELISA | none | carcinomas or lymphomas | escalating doses 1,2,4,8 mg/kg | | Forero-Torres | 20187792 |
| IgM, IgA, IgG to antibody fragment and toxin portion | sandwich ELISA | none | head-neck small cell | escalating doses 100, 200, 330, 500, 700, 930 ug weekly for 4 weeks | baseline, before every infusion and day 56 | MacDonald | 19920898 |
| anti-idiotype and anti-FC-IL2 | bridging and competitive binding ELISA | none | melanoma, neuroblastoma | 2-4 cycles on day 1m2m3 every 4 weeks | most during cycle 1, not signs for boost of response after multiple doses | Hank | 19737959 |
| neutralizing antibodies | cytotoxicity assay | hydroxizine, ranitidine, and paracetamol | mesothelioma, ovarian, pancreatic | 10 days continuous infusion in escalating doses 4, 8, 12, 18, 25 ug/k/d | baseline, day 3,5,8,11 predose and EOI | Kreitman | 19671873 |
| HAHA | ELISA | antihistaminics and paracetamol as premedication | non-hodgkin lymphoma | 4 qw doses | postdose week 4, 12 month 6, 12 | Morschhauser | 19451441 |
| IgM IgG IgA , against scFv, toxin, and VB4-845 | sandwich ELISA | none | head-neck small cell | 2 cycles of 5 consecutive days followed by 23 stop with escalating doses 20, 40, 80, 130, 200, 280 ug | cycle 1 day 1 : 10 min, 30 min, 1hr,2hr,4,6,12, d2, d6,14,21 and 5 of and day 1 of cycle 2 at EOT | MacDonald | 19016010 |
| IgG against MLN2704, nonconjugated Ab, and the DM1 moiety. bridging ELISA measures anti-idiotype | bridging ELISA and sandwich ELISA | none | prostate cancer | 3 doses every 4 weeks | baseline, week 3, 1 week after each dose and 30 days EOT | Galsky | 18362364 |
| nd | ELISA | none | advanced solid tumors | escalating doses , qw, q2w or q3w with or withour loading dose | baseline, 2 timepoints after last infusion | Weiner | 18223225 |
| nd | direct binding assay and competitive binding assay | none | advanced solid tumors | escalating doses q3w | predose, EOI and 5 min, 8h, 7days and 12 days in cycle 1-4 and 21 and 42 days after EOT | Plummer | 17947486 |
| anti-idiotype | bridging ELISA | diphenhydramine, paracetamol as premedication | advanced solid tumors | escalating doses 1-6 mg/kg first as single dose, then 2-5 weeks later weekly doses | baseline, at week 3,6,9 | Delbaldo | 17876527 |
| anti-idiotype | bridging ELISA | none | prostate | two doses on day 0 and 14 in escalating doses | baseline, day 28,35,42 | Oberneder | 16930989 |
| IgG | direct binding ELISA | radiotherapy | glioma | 6 infusions qw 200 mg icm radiotherapy | baseline, day 7, monthly up to 6 months | Ramos | 16575203 |
| nd | MS (surface enhanced laser desorption/ ionization) | none | prostate | intrapatient dose escalations | baseline, day 4, 8 and any day beyween day 15-19 | Morris | 16243819 |
| IgG and IgM | surface plasmon resonance immunoassay | none | colorectal cancer | 4 qw (weekly) doses | weekly | Ritter | 11559561 |
| IgG and IgM | surface plasmon resonance immunoassay | chemotherapy | | | | | |
| IgG and IgM | surface plasmon resonance immunoassay | none | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| nd | ELISA | most people metcolopramide or granistrone, amendement to premedicate with methylprednisolone and in three patients isotretinoine | advanced solid tumors | q3w in escalating doses up to 150 mgm2 | before each infusion, once weekly between courses and at EOT and sign of hemolysis | Awada | 12453862 |
| nd | ELISA | metoclopramide or granisetrone and paracetamol | | q3w up to 125mgm2 | | | |
| nd | ELISA | metoclopramide or granisetrone and paracetamol | | 3 days every 3 weeks, start with 35 mgm2 and escalate with 10 mgm2/day | | | |
| Polyvalent against murine part of MoAb | sandwich ELISA | pretreatment with 3 mg of labeled drug | glioma meningioma | four infusions every four days, in escalating doses 40, 80, 120 mg | screening, day 15 day and monthly up to 6 months | Crombet | 11394532 |
| IgG against murine part of MoAb | sandwich ELISA | pretreatment with 3 mg of labeled drug | epithelial tumors | four infusions every thredays, in escalating doses 50, 100, 200, 300, 400, 500 mg | screening, day 15 and monthly up to 12 months | Crombet | 11279803 |
| IgG and IgM against murine part of MoAb | sandwich ELISA | costimulation with CD28 | B cell lympohoma | singel infusion of 30ug, 270,810 or 1600 | screening, 6 weeks | Manzke | 11251974 |
| IgG against murine and human part of MoAb | sandwich ELISA | none | head-neck small cell | 2 mg microdose followed by singel dose 12 or 52 mg of 186Re-cMab | 5, 10, and 30 min and at 1, 2, 4, 16, and 21 h after injection and 72 and 144 hours | Colnot | 11138685 |
| Neutralizing MoAbs | cytotoxicity assay | hydroxizine, ranitidine as premedication in the last 8 patients. Corticosteroids in CLL patients. | hematologic malignancies | QOD 3 doses in escalating doses, if no ADAs 3 weekly cycles could be continued | 2 min, 1 hour, 4 hours, 12 hours | Kreitman | 10764422 |
| IgG against idiotype | sandwich ELISA | none | advanced solid tumors | q2W for three courses in escalating dose levels from 50-1600 ug | 1, 2 ,4, 24 hours after each administration | Richards | 10232594 |
| murine part | sandwich ELISA | RIT: 131I | renal cell carcinoma | repeated dosing of 10 mg with escalating radioactivity, but only single doses given due to ADAs | weekly | Divgi | 9829736 |
| ADA with lambda light chain | sandwich ELISA | none | multiple myeloma | two cycles of 14 daily infusions in escalating doses of 5,10,20,40 mg/d . Test dose with 10 ug before each cycle. | nd | Van Zaanen | 9722307 |
| murine part | sandwich ELISA | antihistaminics and prednisone as premedication | hodgkin lymphoma | four times every 3-4 days in escalating doses from 1 -64 mgm2 | week 4 | Renner | 9435869 |
| nd | RIA and HPLC | none | advanced solid tumors | single dose of 5 mg labeled antibody administered after test dose of 100 ug | predose, week 2, month 1,3,6 | Wong | 9430476 |
| human and ricin part | RIA | none | B cell lymphoma | continuous 8 day infusion in escalating doses, after test dose of 0,2 mg | nd | Sausville | 7780133 |

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|---|---|---|---|---|---|---|---|
| anti-idiotype | bridging RIA | none | myeloid leukemia | 6 doses per patient over 18 days over 3 to 4 day intervals | weekly 8 times and monthly until 4 months | Caron | 8142644 |
| anti-idiotype | bridging ELISA | none | lung, colon or breast cancer | single escalating doses | baseline, weekly | Goodman | 8382560 |
| nd | solid phase binding immunoassay | none | advanced cancer | qw for 6 weeks | baseline and posttreatment | Sarna | 2319262 |
| nd | ELISA | none | colorectal cancer | first 10µg, then 24h later 100µg, 2wks later 10µg. | baseline and weekly pre-dose for 6 weeks | Robins | 1913661 |
| ELISA measures IgGs | indirect ELISA, Western blot, and Proliferation assay. | IFN-a2B | renal cell carcinoma | day 1,2 every 12 hours. Then 5 days per week for 6 weeks. | | Kirchner | 2222898 |
| ELISA measures IgGs | indirect ELISA, Western blot, and Proliferation assay. | IL2 | | 3qw for 6 weeks | | | |

nd = not described
na = not applicable
RIA= radioimmunoassay
ELISA = enzyme linked immunosorbent assay
RIT = radioimmunotherapy
ADA = anti-drug antibody
HPLC = high performance liquid chromatography
mAb = monoclonal antibody
N= neutral to immune system
I = inhibits immune system
S = stimulates immune system
C = chimeric
H = human
HZ = humanized
P = protein
T = Toxin
M = murine
sc = subcutaneous
it = intratumoral
il = intralymphatic
im = intramuscular
INF = infusion time
ADATYPE = types (immunoglobulin subclass) and characteristics
PK = pharmacokinetics
EOT = end of treatment
HACA = human anti chimeric antibody
HAMA = human anti mouse antibody
HAHA = human anti human antibody
iv = intravenous
ip = intraperitoneal

## SUPPLEMENTARY MATERIAL - METHODS

A PubMed search was performed on November 17th 2015 using the search terms (((((((("Neoplasms"[Mesh] OR cancer* [tiab] OR neoplas* [tiab] OR tumor* [tiab] OR tumour* [tiab] OR carcinom* [tiab] OR adenom* [tiab] OR malignan* [tiab]))))) AND (immunogenicity OR (anti drug antibod* [tiab])))) AND ("Clinical Trials, Phase I as Topic"[Mesh] OR "Clinical Trials, Phase II as Topic"[Mesh] OR "Clinical Trials, Phase III as Topic"[Mesh] OR "Clinical Trials, Phase IV as Topic"[Mesh] OR phase I [tiab] OR phase 1 [tiab] OR phase II [tiab] OR phase 2 [tiab] OR phase III [tiab] OR phase 3 [tiab] OR phase IV [tiab] OR phase 4 [tiab] OR "Clinical Trials, Phase I as Topic"[Mesh] OR "Clinical Trials, Phase II as Topic"[Mesh] OR "Clinical Trials, Phase III as Topic"[Mesh] OR "Clinical Trials, Phase IV as Topic"[Mesh])) NOT vaccine)). Abstracts were scanned and the article was included for review if data on clinical immunogenicity of biological anticancer agents were described. References of the included articles were used to retrieve additional information. Our search yielded 174 results, of which 93 were excluded, leaving 81 for inclusion. Articles were excluded if no data on ADA formation were available or the article included no biological anticancer treatment, no clinical trial, preclinical data only, viral agents or cell therapy.

For 67 monoclonal antibodies, 10 immunotoxins and 4 proteins, data were extracted on ADA formation in terms of the incidence of ADA formation, the consequences, detection of ADAs, and additional risk factors, as described in Appendix Table 1. Quantitative data are based on these clinical trials to provide an up-to-date status of the relevance of ADAs. The incidence was reported as present or absent for the investigated drug, rather than in frequencies because the absolute values are dependent on the used assay, timepoints of sampling and population size and should be interpreted with caution. Additionally, immunogenicity data from 26 EMA or FDA approved biological anticancer agents were extracted from drug reports (European Public Assessment Reports (EPARs, section Immunogenicity), FDA drug reports, section 6.2 Immunogenicity). These data were used only to describe the percentage of ADA positive patients and the clinical relevance for registered and commonly used drugs. For the registered drugs, we believe that the percentage of ADA positive patients is informative since these values were based on a large amount of clinical data. Data of registered agents were not included in the quantitative results from our literature review because (1) no details on detection methods, timepoints, population, co-medication etc. could be retrieved and (2) adding these 26 registered drugs gives a biased view on the overall data on ADA formation. During data collection of the analytical methods, the term bridging ELISA was used for assays with the therapeutic drug as both the capture and detection agent. If this method was used, the detected ADAs were regarded as anti-idiotypic ADAs. The term sandwich ELISA was used for assays with a secondary antibody against an immunoglobulin class (e.g. anti-human IgG antibody). The immunoglobulin subclasses of detected ADAs were deduced from the secondary antibody used (e.g. IgG ADAs when a anti-human IgG detection antibody was used). All other data were reported as in the original article. Statistical analysis was performed using SPSS version 22 (IBM, New York, NY, USA). Logistic regression was used to detect differences in incidence of ADAs between types of antibodies (humanized, human, chimeric and murine) and between immunosuppressing, neutral and immunostimulatory agents. The incidence of ADAs between trials with and without detection of pre-existing ADAs was done using Fisher's exact test.

**Appendix table 1.** Endpoints

| **Endpoint** | Description |
|---|---|
| **Incidence** | Percentage of ADA positive patients |
| **Agent** | Administration route, infusion time, type of agent , target, treatment regimen |
| **Consequences** | Reported effects on pharmacokinetics, efficacy, incidence or severity of toxicity |
| **Detection** | Analytical method, ADA characteristics, time points of sampling and onset of detection |
| **Risk factor** | Immunostimulatory or suppressant effects, concomitant treatment, population size, treated disease |

# Chapter 2

# Combining targeted agents for the treatment of *KRAS* mutant tumors

## Chapter 2.1

**Phase I study of afatinib plus selumetinib in patients with *KRAS*-mutation positive colorectal, non-small cell lung and pancreatic cancer**

*Interim analysis*

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**ABSTRACT**

**Background** Mutations in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene are common in several cancer types and result in a constitutively activated RAS-RAF-MEK-ERK (MAPK) pathway. In these tumors, effects of treatment with MEK inhibitors are limited. Preclinical data showed that this may be caused by intrinsic resistance due to feedback activation of upstream epidermal growth factor receptors (HER) upon MEK inhibition, which not only reactivates the MAPK but also the phosphoinositide 3-kinase (PI3K)-AKT pathway. Based on these data, a phase I clinical trial was initiated with the combination of the MEK inhibitor selumetinib and the pan-HER inhibitor afatinib in patients with *KRAS* mutant and *PIK3CA* wildtype tumors in order to determine the optimal dose and regimen of the combination.

**Methods** In two centers in the Netherlands, patients with *KRAS* mutant and *PIK3CA* wildtype colorectal cancer (CRC), pancreatic cancer and non-small cell lung cancer (NSCLC) received escalating doses of afatinib and selumetinib according to a 3+3 design starting with 25 mg selumetinib bidaily (BID) in a 21 days on/7 days off regimen and 20 mg afatinib once daily (QD) continuously. Extensive blood sampling was performed for pharmacokinetic analyses, and tumor biopsies were taken for pharmacodynamic analyses. Target engagement in tumor biopsies was assessed by immunohistochemistry of pERK and pS6 levels. The study is registered at ClinicalTrials.gov (NCT2450656).

**Results** At data cut-off, 19 patients were enrolled of whom 16 had colorectal cancer, two non-small cell lung cancer and one pancreatic cancer. Dose-limiting toxicities occurred in five patients and consisted of grade 3 diarrhea, decreased appetite, nausea, vomiting and mucositis. The most frequently observed adverse events were diarrhea (95%), skin toxicity (89%) and dyspepsia (32%). The recommended phase 2 dose with continuous afatinib dosing was determined at 20 mg afatinib QD and 25 mg selumetinib BID in a 21 days on/7 days off regimen. Intermittent dosing regimens are currently being explored to allow optimization of exposure and tolerability. At all doses, modulation of pERK and pS6 was confirmed in on-treatment biopsies (mean H-score change -52% and -39%). However, clinical efficacy was limited with the best response being disease stabilisation for 196 days in a patient with CRC but no objective tumor regressions.

**Conclusion** We demonstrated that it is clinically feasible to combine afatinib and selumetinib in patients with *KRAS* mutant tumors, although single agent full doses cannot be applied due to dose-limiting toxicities. In the ongoing study, intermittent dosing strategies are explored to optimize exposure and tolerability.

## INTRODUCTION

The RAS protein plays a pivotal role in the regulation of cell proliferation, survival and differentiation. Among the different subtypes of RAS, being NRAS, HRAS and KRAS, the KRAS subtype has the strongest and widest regulatory effects in cells[1]. Activating mutations in KRAS are frequently observed in human cancers and are associated with high rates of cancer cell proliferation[1,2]. Whereas RAS is normally activated by growth factors that bind to the extracellular protein HER, activating KRAS mutations can cause a constant and independent stimulation of cell proliferation. Mutations in the KRAS protein occur as frequently as 45% in colorectal cancer (CRC), 35% in non-small cell lung cancer (NSCLC) and 90% in pancreatic cancer[1]. In these tumor types, KRAS mutations have been associated with poor responses to standard-of-care treatment. Approaches to inhibit KRAS signaling include targeting KRAS directly or targeting proteins activated by KRAS, such as RAF, MEK or ERK. However the outcomes of these strategies have been disappointing in the preclinical and clinical setting[2–5]. As a result, no targeted treatment options are currently available for this group of patients indicating a high medical need for new therapeutic strategies. Pre-clinical data show that intrinsic resistance upon MEK inhibition is due to feedback activation of upstream epidermal growth factor receptors (HER). This overexpression reactivates the MAPK but also the phosphoinositide 3-kinase (PI3K)-AKT pathway[6]. *In vitro*, cell growth of KRAS mutant cell lines could be completely suppressed by inhibiting the proteins MEK and HER. Among various combinations, the combination of the MEK inhibitor selumetinib and the potent irreversible inhibitor of multiple HER family kinases afatinib (*in vitro* $IC_{50}$ values of 0.5 nM, 14 nM and 1 nM against the human catalytic domains of HER1, HER2 and HER4) showed the strongest anti-tumor effects. The activity of the combination was subsequently confirmed in mice with *KRAS* mutated NSCLC.[2]

The unmet medical need and the promising pre-clinical results provided a strong rationale to evaluate the combination of afatinib and selumetinib in patients with *KRAS*m CRC, NSCLC and pancreatic cancer. In these patients, absence of *PIK3CA* mutations was required to avoid treatment resistance via activation of signaling proteins downstream of PI3K[6]. In this phase I trial, we investigated the safety, tolerability and preliminary anti-tumor activity of afatinib and selumetinib in order to determine the optimal dose (recommended phase II dose) and regimen.

## METHODS

### Patient population

This investigator-initiated, multi-center, open-label, phase I dose-escalation study enrolled patients at two sites in the Netherlands. Adult patients with histologically- or cytologically-confirmed advanced CRC, NSCLC or pancreatic cancer were enrolled on the basis of a documented *KRAS* mutation in exon 2, 3 or 4, and *PIK3CA* wildtype status. Eligibility criteria included: Eastern Cooperative Oncology Group (ECOG) performance status of ≤2, life expectancy of ≥3 months, measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1, adequate bone marrow (absolute neutrophil count ≥1.5 x $10^9$/L, platelets ≥100 x $10^9$/L, hemoglobin ≥6.0 mmol/L), hepatic (total bilirubin ≤1.5 x upper limit of normal [ULN], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 x ULN), and renal (serum creatinine ≤1.5 x ULN) functions. Radiotherapy, immunotherapy, chemotherapy or any treatment with investigational medication within four weeks prior to study treatment were not allowed, and patients with a history of other primary malignancies were excluded. Additional exclusion criteria included symptomatic or untreated leptomeningeal disease, symptomatic brain metastasis, history of interstitial lung disease or pneumonitis, history of retinal vein occlusion, and prior therapy containing targeted drug combinations known to interfere with EGFR, HER2, HER3, HER4 or MAPK- and PI3K-pathway components, including PI3K, AKT, mTOR, BRAF, MEK and ERK. The study (ClinicalTrials.gov identifier: NCT2450656) was conducted in accordance with guidelines for Good Clinical Practice as defined by the International Conference on Harmonization. Regulatory authorities and the institutional review boards approved the study protocol and all amendments, and all patients gave written informed consent, per Declaration of Helsinki recommendations. In December 2016, the protocol was amended to restrict inclusion to patients with NSCLC only, based on signs of preferential activity as further discussed in the discussion section. The study was funded by an unrestricted grant from AstraZeneca Inc. and Boehringer Ingelheim Inc., which also supplied the investigational drugs.

### Study design and procedures

Patients were treated at different dose-levels of orally administered afatinib and selumetinib in cycles of 28 days. The starting doses were chosen based on previous data from single agent phase I studies with both compounds, also taking into account a potential synergy in toxicities. Dose-level 1 consisted of 20 mg afatinib once daily (QD) continuously, which is 50% of its recommended dose as single agent, and 25 mg selumetinib twice daily

(BID) administered on the first 21 days of each 28-day cycle, which is 33% of its recommended dose as single agent. First, selumetinib was escalated according to a classical 3+3 design with fixed maximum escalation increments. Dose-escalation decisions were based on safety evaluation of all evaluable patients, performed after completion of the first treatment cycle. Patients were considered evaluable for toxicity if at least one cycle of study treatment was completed, with the minimum safety evaluation and drug exposure (≥75% of the planned doses of selumetinib and afatinib) or if dose-limiting toxicity (DLT) had occurred during the first cycle. If one out of three patients experienced DLT, the number of patients treated at that dose-level was expanded to a maximum of six. Dose-escalation continued until a dose-level was reached at which no more than one out of six patients experienced DLT during the first 28 days of treatment, provided that the single agent recommended dose was not exceeded. Subsequently, afatinib was escalated with fixed increments of 10 mg following the same dose-escalation rules. Upon assessment of the optimal dose of the two-drug combination in this regimen, an intermittent dosing regimen was investigated which consisted of 5 days on/2 days off for both drugs during 28-day cycles with the aim of optimizing drug exposure and improving tolerability. Patients were to continue study treatment until disease progression, unacceptable toxicity despite supportive measures and dose modifications, or investigator's or patient's decision to discontinue. Safety was monitored throughout the treatment by physical examination, laboratory assessments, electrocardiography, ophthalmic evaluation, left ventricular ejection fraction monitoring by multigated acquisition scan (MUGA) and recording adverse events. Adverse events were graded according to Common Terminology Criteria for Adverse Events version 4.0. DLT was defined as an adverse event or laboratory abnormality occurring within the first treatment cycle meeting at least one of the criteria described in supplementary table S1. Radiologic tumor measurements were performed using computed tomography (CT) scans at baseline and every six weeks throughout the study. After a protocol amendment, CT scans were performed every eight weeks. Tumor response was evaluated according to RECIST version 1.1[7]. Patients were evaluable for anti-tumor activity if at least one on-treatment radiologic evaluation was performed.

**Pharmacokinetic and pharmacodynamic analyses**

For pharmacokinetic analyses, serial blood samples for plasma concentration analysis were obtained from all patients in cycle 1 before and 1, 1.5, 2, 3, 4, 6, 8, 24, 72, 144, 336 and 456 hours after the first dose. On day 1 of cycle 2, blood samples were drawn before and 1, 2, 3, 4, 6, 8 and 24 hours after administration. Plasma was isolated and stored at -80°C until analysis. Plasma samples were assayed using a validated high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method. Briefly, selumetinib and afatinib were extracted from plasma by protein precipitation with a mixture of acetonitrile/methanol (1:1 v/v). Compounds were chromatographically separated using a Waters Xbridge BEH Phenyl column (50 x 2.1 mm ID, 5 µm particle size) and detection was performed using an API4000 tandem mass spectrometer equipped with a turbo ion spray interface, operating in the positive ion mode. Transitions from m/z 486 to 371 and m/z 459 to 397 were monitored for the detection of afatinib and selumetinib, respectively. Stable labelled internal standards were used for the quantification. The lower and upper limits of quantification were respectively 0.5 and 50 ng/mL for afatinib, and 5 and 500 ng/mL for selumetinib. Pharmacokinetic parameters were calculated in R using an in-house developed package for non-compartmental pharmacokinetic analyses (version 1.3)[8]. For pharmacodynamic analyses, tumor biopsies were taken before treatment, in the second week of treatment and upon treatment discontinuation. Phosphorylated (p) ERK and ribosomal pS6 (pS6-r) levels were measured by validated immunohistochemistry (IHC) staining methods and semi-quantitative H-scores (percentage of positive cells (0–100) multiplied by staining intensity (0–3)) were assessed by an independent pathologist who was blinded for sample identification. Tumor biopsy samples were fixed in formalin for 16–24 hours and embedded in paraffin subsequently. Immunohistochemistry of formalin-fixed paraffin-embedded tumor samples was performed on a BenchMark Ultra autostainer (Ventana Medical Systems). Briefly, paraffin sections were cut at 3 µm, heated at 75°C for 28 minutes and deparaffinised with EZ prep solution (Ventana Medical Systems). Heat-induced antigen retrieval was carried out using Cell Conditioning 1 (CC1, Ventana Medical Systems) at 95°C for 32 and 64 minutes, for pS6-r and pERK1/2, respectively. pS6-r was detected using clone D68F8 (1:1000 dilution, 32 minutes at room temperature, Cell Signalling) and p-p44/42 MAPK (pERK1/2) (Thr202/Tyr204) using clone D13.14.4E (1:400 dilution, one hour at room temperature, Cell Signalling). pERK was detected using the UltraView Universal DAB Detection Kit (Ventana Medical Systems), while detection of pS6-r was performed using the OptiView DAB Detection Kit (Ventana Medical Systems). Slides were counterstained with hematoxylin.

**Statistical analysis**

Pharmacokinetics, pharmacodynamics, safety and tumor response data were reported descriptively. The statistical significance of pERK/pS6-r modulation during treatment was calculated using a paired students' T-test. A linear regression analysis was performed to assess the correlation between area under the plasma concentration-time curve (AUC) of selumetinib and afatinib, assessed on cycle 1 day 1, and modulation of pERK.

## RESULTS

### Patient characteristics

At data cut-off (15 May 2017), 19 patients were enrolled onto this study between July 2015 and May 2017; 16 patients (84%) with CRC, two with NSCLC (11%) and one (5%) with pancreatic cancer. The majority of patients had *KRAS* exon 2 mutations. The most frequently reported mutations were p.G12D (n=8) and p.G12C (n=5). Also, exon 2 p.G12A, p.G12V and p.G13D mutations (n=1 each), exon 3 p.Q61R (n=2) and exon 4 p.A146V (n=1) mutations were reported. Most patients were pretreated with at least two prior lines of antineoplastic therapy for advanced disease (table 1). Fifteen patients were evaluable for toxicity; four patients were considered not evaluable due to early clinical deterioration or patient refusal. At data cut-off, one patient was still ongoing after 2.2 months (figure 3), and 18 patients had discontinued treatment due to progressive disease (n=12), adverse events (n=4) or clinical deterioration (n=2).

### Dose finding

A total of 15 patients was evaluable for DLT. At the initial dose-level, no DLTs occurred in the three included patients. Therefore, doses of selumetinib were escalated to 50 mg BID with the same dose of afatinib 20 mg QD. At this dose-level, two out of the first five patients experienced DLTs, being grade 3 diarrhea and nausea/vomiting in one patient, and diarrhea and dehydration in the other patient. The previous dose-level was expanded to six patients. One patient experienced DLTs being grade 3 increased diarrhea and decreased appetite, which indicated that the highest dose for selumetinib was 25 mg BID in this combination. Subsequently, afatinib was escalated to 30 mg QD which caused DLTs in two out of four patients consisting of grade 3 mucositis and dehydration, which rendered this dose-level intolerable (figure 1). Consequently, the established maximum tolerated dose-level with continuous afatinib dosing consisted of 30 mg afatinib QD plus 25 mg selumetinib BID. To explore if further dose-escalation was possible when the drugs were administered intermittently, a 5 days on/2 days off regimen was initiated which was ongoing at data cut-off.

### Safety

Study treatment-related adverse events were reported in all patients, with the most common being diarrhea (95%), skin toxicity (89%) and dyspepsia (32%). Skin toxicity and gastrointestinal toxicity developed mostly

**Table 1.** Patient and disease characteristics at baseline. *Abbreviations: ECOG PS, Eastern Cooperative Oncology performance status; KRAS, Kirsten rat sarcoma viral oncogene homolog.*

| | Patients (n =19) |
|---|---|
| Sex, n (%) | |
| Female | 8 (42%) |
| Male | 11 (58%) |
| Age, median (range), years | 65 (51-77) |
| Tumor types, n (%) | |
| Colorectal | 16 (84%) |
| Non-small cell lung | 2 (11%) |
| Pancreatic | 1 (5%) |
| ECOG PS, n (%) | |
| 0 | 15 (79%) |
| 1 | 4 (21%) |
| Number of prior treatment lines, n (%) | |
| 1 | 3 (16%) |
| 2 | 5 (26%) |
| ≥ 3 | 11 (58%) |
| KRAS mutation, n (%) | |
| Exon 2 | 16 (84%) |
| p.G12D | 8 (42%) |
| p.G12C | 5 (26%) |
| p.G12A | 1 (5%) |
| p.G12V | 1 (5%) |
| p.G13D | 1 (5%) |
| Exon 3 | 2 (10%) |
| p.Q61R | 2 (10%) |
| Exon 4 | 1 (5%) |
| p.A146V | 1 (5%) |

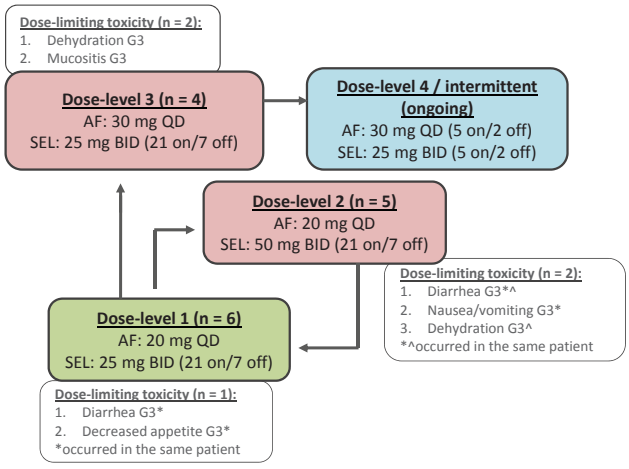


**Figure 1**. Overview of dose-levels and dose-limiting toxicities (DLTs). *Abbreviations: n, number of patients; AF, afatinib; SEL, selumetinib; QD, once daily; BID, twice daily; G, grade.*

within the first weeks of treatment (table 2). Skin toxicity mainly included acneiform rash and dry skin and was limited to grade 1-2 in dose-level 1 and 2. Supportive care, including minocycline and cetomacrogole cream or corticosteroid cream, was sufficient to manage skin toxicity in most patients. One grade 3 rash occurred in dose-level 3 which resulted in treatment discontinuation. The most frequent grade 3 events were diarrhea (n=5, 16%), dehydration (n=4, 21%), nausea, anorexia and hypophosphatemia (each n=2, 11%). The incidence of grade 3 toxicities was clearly higher in dose-levels 2 and 3, at which ten grade 3 events occurred, compared to dose-level 1, at which four patients had grade 3 events. In total, five patients (26%) required a dose-interruption because of grade 3 events including diarrhea (n=3), dehydration (n=2), rash, mucositis and nausea (each n=1). Finally, four patients (21%) discontinued due to grade 2 diarrhea (n=1), grade 3 diarrhea (n=2) or grade 3 dehydration (n=1). All toxicities resolved to grade 1 or less upon interruption or discontinuation of study treatment. Retinopathy and ocular neurosensory detachment were observed in two patients at dose-level 2, in which the highest doses of selumetinib were given, but were limited to grade 1-2 without treatment interruptions. Retinopathy was completely reversible, whereas the neurosensory detachment was grade 1 at the last follow-up. Creatine kinase elevation was observed in two patients at dose-levels 1 and 2 and was also reversible without treatment interruptions.

**Pharmacokinetic analysis**

Pharmacokinetic parameters after the first dose and at steady-state are summarized for each dose-level in table 3. Selumetinib exposure increased dose-proportionally with moderate inter-patient variability. For afatinib, a three-fold increase in exposure was observed in the patients treated with 30 mg compared to the 20 mg cohorts. Taking into account the high inter-individual variability, no conclusions on dose-proportionality can be drawn at the moment. In line with its long half-life of 37 hours[9], the mean afatinib area under the plasma concentration-time curve from time 0 to 24 hours ($AUC_{0\text{-}24h}$) increased approximately two-fold from cycle 1 day 1 to steady

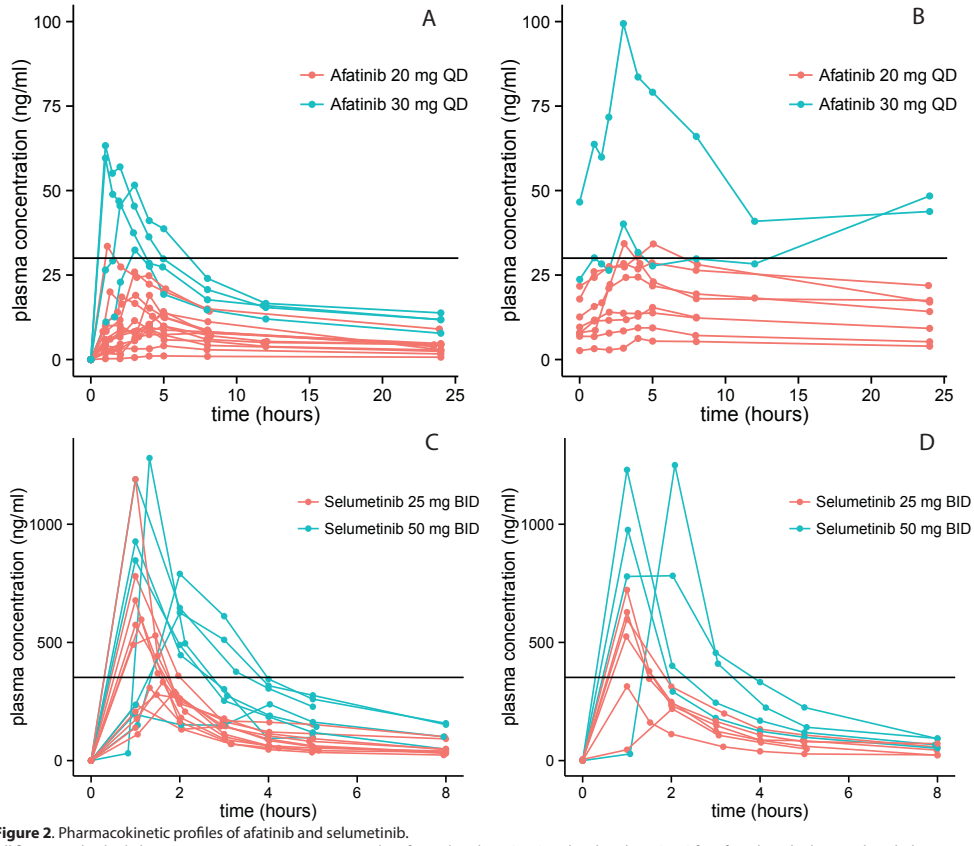


**Figure 2**. Pharmacokinetic profiles of afatinib and selumetinib.
*All figures: Individual plasma concentration time curves per dose for cycle 1 day 1 (A, C) and cycle 2 day 1 (B, D) for afatinib and selumetinib with the respective target levels (----) of 30 ng/mL and 352 ng/mL, based on single agent use.*

state. The plasma-concentration time curves of individual patients show that the target levels of 30 ng/ml for afatinib, based on preclinical proliferation experiments (unpublished data Boehringer Ingelheim) and clinically active plasma concentrations[10], and 352 ng/ml for selumetinib[11] have been reached in all patients treated at the highest doses, although not during the full dosing interval (figure 2).

### Anti-tumor activity

Eleven patients were evaluable for anti-tumor activity; seven patients did not reach the first radiological evaluation after eight weeks of study treatment due to clinical deterioration (n=4) or toxicity (n=3).

**Table 2.** Adverse events, regardless of treatment, occurring in ≥ 10% of patients.
*Abbreviations: CPK, creatine phosphokinase* Some patients had multiple skin toxicities and were only counted once for the category of any skin toxicity.*

| | Dose-level 1 (n =7) | | | | Dose-level 2 (n = 7) | | | | Dose-level 3 (n = 5) | | | | **Total (n = 19)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Afatinib QD*<br>*Selumetinib BID* | *20 mg*<br>*25 mg* | | | | *20 mg*<br>*50 mg* | | | | *30 mg*<br>*25 mg* | | | | | | | |
| **Adverse Event, n (%)** | ***Grade 1/2*** | | ***Grade 3*** | | ***Grade 1/2*** | | ***Grade 3*** | | ***Grade 1/2*** | | ***Grade 3*** | | **Grade 1/2** | | **Grade 3** | |
| Diarrhea | 5 | 26% | 1 | 5% | 3 | | 4 | 21% | 5 | 26% | | | **13** | **68%** | **5** | **26%** |
| Any skin toxicity* | 6 | 32% | | | 6 | 32% | | | 4 | 21% | 1 | 5% | **16** | **84%** | **1** | **5%** |
| *Rash acneiform* | 4 | 21% | | | 6 | 32% | | | 4 | 21% | 1 | 5% | **14** | **74%** | **1** | **5%** |
| *Dry skin* | 2 | 11% | | | 1 | 5% | | | 4 | 21% | | | **7** | **37%** | | |
| *Erosive skin* | 1 | 5% | | | | | | | | | | | **1** | **5%** | | |
| *Eczema* | | | | | 1 | 5% | | | | | | | **1** | **5%** | | |
| *Skin infection* | | | | | 2 | 11% | | | | | | | **2** | **11%** | | |
| Edema | 2 | 11% | | | 2 | 11% | | | 3 | 16% | | | **7** | **37%** | | |
| Dyspepsia/reflux | 1 | 5% | | | 2 | 11% | | | 3 | 16% | | | **6** | **32%** | | |
| Mucositis | | | | | 2 | 11% | | | 2 | 11% | 1 | 5% | **4** | **21%** | **1** | **5%** |
| Anorexia/dysgeusia | 1 | 5% | 1 | 5% | | | | | 3 | 16% | 1 | 5% | **4** | **21%** | **2** | **11%** |
| Eye toxicity | 2 | 11% | | | 2 | 11% | | | 1 | 5% | | | **5** | **26%** | | |
| *Blurred vision* | 2 | 11% | | | 1 | 5% | | | | | | | **3** | **16%** | | |
| *Neurosensory detachment* | | | | | 1 | 5% | | | | | | | **1** | **5%** | | |
| *Retinopathy* | | | | | 1 | 5% | | | | | | | **1** | **5%** | | |
| *Photosensitivity* | 1 | 5% | | | | | | | | | | | **1** | **5%** | | |
| *Dry eyes* | | | | | | | | | 1 | 5% | | | **1** | **5%** | | |
| Dehydration | | | 1 | 5% | | | 2 | 11% | | | 1 | 5% | | | **4** | **21%** |
| Fatigue | | | | | 1 | 5% | | | 1 | 5% | 1 | 5% | **2** | **11%** | **1** | **5%** |
| Nausea | | | | | 2 | 11% | 1 | 5% | | | 1 | 5% | **2** | **11%** | **2** | **11%** |
| Vomiting | | | | | 2 | 11% | | | | | 1 | 5% | **2** | **11%** | **1** | **5%** |
| Pain | 1 | 5% | | | 1 | 5% | | | 1 | 5% | | | **3** | **16%** | | |
| Hypophosphatemia | | | | | 1 | 5% | 2 | 11% | | | | | **1** | **5%** | **2** | **11%** |
| CPK elevation | 1 | 5% | | | 1 | 5% | | | | | | | **2** | **11%** | | |
| Fever | 1 | 5% | | | | | | | | | 1 | 5% | **1** | **5%** | **1** | **5%** |
| Epistaxis | 1 | 5% | | | | | | | 1 | 5% | | | **1** | **5%** | **1** | **5%** |
| Hypertension | | | 1 | 5% | | | | | | | 1 | 5% | **1** | **5%** | **1** | **5%** |
| Neuropathy | | | | | 1 | 5% | 1 | 5% | | | | | **1** | **5%** | **1** | **5%** |
| Dry mouth | | | | | 1 | 5% | | | 1 | 5% | | | **2** | **11%** | | |

**Table 3.** Pharmacokinetic parameters of afatinib and selumetinib at day 1 and steady-state. *Data are listed as geometric mean. $AUC_{0\text{-}24}$ data for the combined 20 mg afatinib dose-levels and 25 mg selumetinib dose-levels are given as mean (CV%) and [95% confidence interval].*

| **Dose-level** | **1** | **2** | **3** | ***Afatinib*** | ***Afatinib*** | ***Selumetinib*** | ***Selumetinib*** |
|---|---|---|---|---|---|---|---|
| *Afatinib OD* | 20 mg | 20 mg | 30 mg | All 20 mg doses | All 30 mg doses | All 25 mg doses | All 50 mg doses |
| *Selumetinib BID* | 25 mg | 50 mg | 25 mg | | | | |
| **Afatinib** *Cycle 1 Day 1* | | | | | | | |
| *Mean* | n = 7 | n = 7 | n = 4 | n = 14 | n = 4 | - | - |
| $C_{max}$ (ng/mL) | 15.4 | 13.1 | 51.7 | 14.3 | 51.7 | | |
| $T_{max}$ (h) | 3.7 | 3.4 | 2.0 | 3.5 | 2.0 | | |
| $AUC_{0\text{-}24h}$ (ng*h/mL) | 162 | 165 | 465 | 161 (53%) [19-340] | 465 (21%) [321-535] | | |
| **Afatinib** *Cycle 2 Day 1* | | | | | | | |
| *Mean* | n = 4 | n = 4 | n = 1 | n = 8 | n = 1 | - | - |
| $C_{max}$ (ng/mL) | 25.7 | 16.0 | 99.4 | 20.8 | 99.4 | | |
| $T_{max}$ (h) | 4.8 | 3.5 | 3 | 4.1 | 3.0 | | |
| $AUC_{0\text{-}24h}$ (ng*h/mL) | 468 | 251 | 1312 | 359 (52%) [109-597] | 1312 (0%) | | |
| **Selumetinib** *Cycle 1 Day 1* | | | | | | | |
| *Mean* | n = 7 | n = 7 | n = 4 | - | - | n = 13 | n = 7 |
| $C_{max}$ (ng/mL) | 472.1 | 836.6 | 2509.8 | | | 525.8 | 836.6 |
| $T_{max}$ (h) | 1.3 | 1.3 | 1 | | | 1.3 | 1.3 |
| $AUC_{0\text{-}8h}$ (ng*h/mL) | 1125 | 2207 | 620 | | | 1202 (38%) [700-1949] | 2207 (29%) [1126-3283] |
| **Selumetinib** *Cycle 2 Day 1* | | | | | | | |
| *Mean* | n = 4 | n = 4 | n = 2 | - | - | n = 6 | n = 4 |
| $C_{max}$ (ng/mL) | 413.8 | 1059.5 | 675.0 | | | 500.8 | 1059.5 |
| $T_{max}$ (h) | 1.3 | 1.5 | 1.0 | | | 1.2 | 1.5 |
| $AUC_{0\text{-}8h}$ (ng*h/mL) | 1023 | 2377 | 1468 | | | 1171 (36%) [607-1590] | 2377 (19%) [1856-2943] |

Of one patient, the first tumor evaluation was not yet available at the time of data cut-off. Out of the evaluable patients, seven achieved stable disease and four had progressive disease on their first radiologic evaluation scan. The largest tumor regression of -16% was seen in a NSCLC patient treated on dose-level 3. All other patients who were evaluated with stable disease had changes of -7% to 15% in tumor volume (figure 3). The overall median treatment duration was 56 days (range 11–196), with four CRC patients with a disease stabilization ≥112 days (4 months). Across the differential dose-levels, median treatment duration was the longest on dose-level 1 (63 days, range 20-112), followed by dose-level 2 (50 days, range 11-196) and 3 (49 days, range 26-63) (figure 4).

**Pharmacodynamic analyses**

Tumor biopsies were obtained from all patients in the study. Stainings were evaluable for 17 patients, of whom six had evaluable pre- and on-treatment biopsies which allowed assessment of treatment effects. The other biopsies could not be obtained or were not evaluable because of low percentages of tumor cells. The median decrease of pERK was –52% ($p$=0.02*)* and for pS6-r –39% ($p$=0.2*)*, indicating adequate inhibition of MEK and HER signaling by the combination treatment (figure 5). Modulation of pERK was achieved in all patients at all doses and matched with modulation of pS6-r in five out of six patients. The degree of pERK modulation significantly correlated with the AUC of selumetinib as assessed on cycle 1 day 1 ($R^2 = 0.85$, $p < 0.01$).

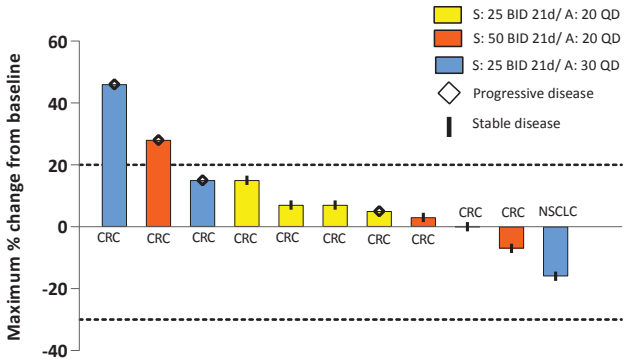


**Figure 3**. Maximum percentage change in sum of target lesion size from baseline, by dose-level, including response evaluation by RECIST.
*Abbreviations: A, afatinib; S, selumetinib; QD, once daily; BID, twice daily; CRC, colorectal cancer; NSCLC, non-small cell lung cancer.*

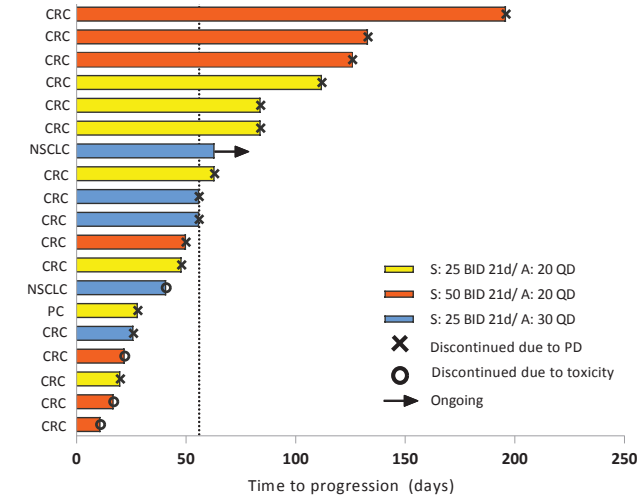


**Figure 4**. Swimmer plot of treatment duration, by dose-level.
*Abbreviations: A, afatinib; S, selumetinib; QD, once daily; BID, twice daily; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; PC, pancreatic* cancer; *PD, progressive disease; d, days. Symbols at the end of each bar represent the reason for end of treatment for each individual patient. The dotted line represents the median time-on-treatment of 54 days.*

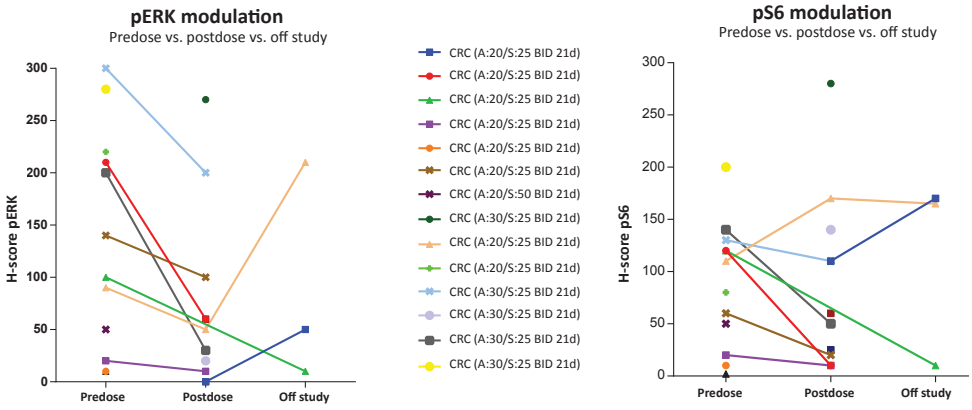


**Figure 5.** Pharmacodynamic effects of afatinib and selumetinib. Individual pERK and p-S6r intensity scores (H-scores) of tumor biopsies at baseline, on-treatment (day 15) and upon study discontinuation are presented as determined by immunohistochemistry staining. Mean H-score decreases were -52% for pERK and -39% for pS6-r.
*Abbreviations: CRC, colorectal cancer; PC, pancreatic cancer; NSCLC, non-small cell lung cancer; A, afatinib; S, selumetinib; BID, twice daily; d, days.*

## DISCUSSION

In this clinical study we demonstrated for the first time that the MEK inhibitor selumetinib combines safely with the multiple-HER inhibitor afatinib, although not at full single agent doses because of dose-limiting toxicities. The observed toxicities are in line with the monotherapy toxicity profiles of both agents. Yet, the use of high doses was limited by overlapping toxicities such as diarrhea and dehydration as a result.

In parallel with this trial, two other studies with combined MEK and pan-HER inhibition were conducted[12,13]. Emerging unpublished data from these trials, also described in this thesis, showed a trend towards preferential activity in NSCLC compared to CRC and pancreatic cancer. These clinical observations are also supported by previous clinical studies that show that MEK inhibition added to second line docetaxel in patients with NSCLC can lead to improved responses, whereas in CRC the addition of a MEK inhibitor to second line irinotecan did not result in clinical benefit[14,15]. Although the trend towards higher efficacy in NSCLC could not be confirmed in this trial due to the low number of NSCLC patients enrolled, evidence was considered strong enough to amend the protocol and include only patients with NSCLC starting from December 2016.

At the time of this interim-analysis, only one patient with NSCLC had been included in dose-level 3. Although this patient had a tumor regression of -16%, treatment was discontinued due to intolerable toxicity. This illustrates what has been observed throughout the study; the combination of afatinib and selumetinib is potentially effective at higher doses, yet reaching these doses seems clinically not feasible in the regimens that have been applied so far. The intermittent regimens that are currently explored may allow improvement of the efficacy to toxicity ratio.

Both for selumetinib and afatinib, pharmacokinetic parameters are largely in line with previous studies[9,11]. Our data show no signs of pharmacokinetic interactions between afatinib and selumetinib although the study design does not enable us to completely rule out a drug-drug interaction. The recommended phase 2 dose with continuous afatinib administration was found to be 25 mg BID selumetinib 21 days out of 28 days and 20 mg afatinib continuously which is 33% and 50% of their monotherapy doses. At these doses, individual plasma concentration-time curves show that relatively low plasma levels and exposures are reached. For selumetinib, a concentration of 352 ng/ml is needed for 50% of pERK[11] inhibition in peripheral blood mononuclear cells, as reported in a previous phase I trial. In this study, at the RP2D in combination with afatinib, this concentration was reached for only 17% of each 12-hour dosing interval. For afatinib, the concentration needed for 50% inhibition of cell proliferation in preclinical setting was determined as approximately 30 ng/ml, which is also supported by clinical efficacy in patients in whom these plasma concentrations were reached[10]. This target-concentration was consistently reached only in patients treated with afatinib 30 mg, which was not tolerable in combination with selumetinib in the applied schedule. Although the relevance of target levels in this combination setting is unsure because these target levels are based on single-agent use, we assumed that the probability of response increases when the target levels of both drugs are more consistently reached. Based on this, it was decided to explore other regimens with the aim of increasing the plasma levels and exposure, starting with a 5 days on/2 days off regimen with doses of 30 mg afatinib and 25 mg selumetinib. This dose-level is currently ongoing.

Despite relatively low plasma levels, we did observe pharmacodynamic effects in on-treatment biopsies that were taken in the second week of treatment. The intra-tumoral levels of pERK and pS6 decreased by on average 52% and 39%, respectively. This indicates that relevant MEK and pan-HER inhibition is reached during the treatment. Moreover, pERK modulation correlated with exposure to selumetinib. However, this did not translate into objective clinical responses. The best responses were 16% tumor regression in one patient with NSCLC and disease stabilization for seven months in a patient with colorectal cancer. It is unknown if these patients had relevant modulation of pERK and pS6 because no paired biopsies were available.

The poor correlation between pathway modulation and clinical response could be due to a variety of mechanisms. First, inter-metastasis heterogeneity may play an important role because the pharmacodynamic analyses were based on a single lesion only. Insight in the relevance of this mechanism may be obtained by correlating the radiological response of the biopsied lesion to the pERK/pS6 modulation. For this study, data were not sufficient to perform these analyses. However, in the lapatinib-trametinib combination study, described in chapter 2.3, the pERK modulation indeed correlated better with response of the biopsied lesion than to the overall clinical response.

Secondly, the observed pathway modulation may be transient or insufficient, meaning that resistance mechanisms occur shortly after the on-treatment biopsy which was performed in the second week. Several

resistance mechanisms could play a role. Corcoran *et al.* reported that upon MEK inhibition, resistance occurs via expression of anti-apoptotic proteins Bcl-xL[16]. Addition of the Bcl-xL inhibitor navitoclax induced apoptosis in MEK inhibitor-resistant cells. Potentially, this resistance mechanism may play a role in dual MEK/pan-HER inhibition too. Therefore, addition of a third agent could be of interest, if clinically feasible. Furthermore, Burgess *et al.* showed that *KRAS* copy numbers, *KRAS* expression levels and the ratio *KRAS* mutant to wildtype can explain resistance to MEK inhibition[17] and Sun *et al.* showed that high tumor-expression of HER2/HER3 at baseline increased the probability of response on MEK and pan-HER inhibition[2]. Conversely, upregulation of HER2/HER3 during treatment could theoretically cause treatment resistance via pathway reactivation. For patients treated in our trial, tumor material is being used for DNA and RNA sequencing with the aim of exploring the true mechanisms of response and resistance. The final results of these analyses are expected soon and may give us new insights into rational use of MEK and pan-HER inhibitors in the clinic. These results may also reveal differences in sensitivity of NSCLC versus the other tumor types, and the impact of the different *KRAS* mutations on response and resistance.

To improve treatment options for patients with a *KRAS* mutation, triple combinations could be effective to overcome resistance mechanisms. Potentially, synergistic effects may allow the use of relatively low doses of all agents, which is supported by preliminary data of MEK and pan-HER inhibition in *KRAS*m CRC organoids (unpublished). The same unpublished data show that the dual combination might provide an effective treatment option for patients with *KRAS* wildtype tumors. Although standard treatment options exist for *KRAS* wildtype CRC, NSCLC and pancreatic cancer, targeted combinations may improve current responses. However, before this application may be explored, first the optimal dosing regimen with most beneficial efficacy-to-toxicity ratio should be defined based on the final results of this ongoing phase I study.

In conclusion, we showed that afatinib and selumetinib can be combined in patients with *KRAS* mutant tumors, although single agent full doses cannot be applied due to dose-limiting toxicities. The optimal regimen of this combination is to be determined in the ongoing study.

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## SUPPLEMENT

**Table S1.** Criteria for defining dose-limiting toxicities. *Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ULN, upper limit of normal; LLN, lower limit of normal; DLT, dose limiting toxicity.*

| Toxicity | DLT definition |
|---|---|
| Hematologic | • Grade 4 neutropenia for ≥5 days<br>• Grade ≥3 febrile neutropenia<br>• Grade 4 anemia<br>• Grade 4 thrombocytopenia |
| Non-hematologic | • AST >5X ULN OR ALT >3X ULN and bilirubin >2X ULN (after exclusion of disease progression and/or bile duct obstruction)<br>• Grade ≥4 rash, hand-foot syndrome or photosensitivity<br>• Grade 3 rash, hand-foot syndrome or photosensitivity for more than 7 days despite adequate supportive treatment.<br>• Grade ≥3 nausea, vomiting or diarrhea in the presence of maximal supportive care<br>• Grade ≥2 peripheral sensory or motor neuropathy<br>• Grade ≥3 clinically significant toxicity related to study treatment, other than those listed above, with the following exceptions:<br>• Electrolyte disturbances that respond to correction within 24 hours<br>• Grade 3 hypertension that is adequately controlled by the addition of up to two additional antihypertensive medications<br>• Grade 3 pyrexia that does not result in study discontinuation |
| Cardiac | • Ejection fraction <55% with an absolute decrease of >10% from baseline with confirmation within 14 days |
| Other | • Inability to receive ≥75% of scheduled doses in treatment period due to toxicity related to study treatment<br>• Grade 2 or higher toxicity that occurs beyond 28 days which in the judgment of the investigator is a DLT |

## Chapter 2.2

**Phase I study of dacomitinib plus PD-0325901 in patients with *KRAS*-mutation positive colorectal, non-small cell lung and pancreatic cancer**

*Interim analysis*

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**ABSTRACT**

**Background** Mutations in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene are common in several cancer types and result in a constitutively activated Ras-Raf-MEK-ERK (MAPK) pathway. In these tumors, existing treatment options, including inhibition of MEK, have limited efficacy. Preclinical work showed that this is due to intrinsic resistance due to feedback activation of upstream epidermal growth factor receptors (HER) upon MEK inhibition. Based on these data, a clinical trial was started in which the combination of the MEK inhibitor PD-0325901 and the pan-HER inhibitor dacomitinib was administered to patients with *KRAS* mutated tumors.

**Methods** In this multicenter, open-label, phase I dose-escalation study we investigated the combination of the human epidermal growth factor receptor (HER) family inhibitor, dacomitinib, plus a selective MEK1/2 inhibitor, PD-0325901, in patients with *KRAS* mutant (*KRAS*m) colorectal cancer (CRC), non-small cell lung cancer (NSCLC) and pancreatic cancer. Patients received escalating doses of once daily (QD) oral dacomitinib and twice daily (BID) oral PD-0325901 (21 days on/7 days off) to determine the recommended phase II dose (RP2D), starting with 30 mg QD dacomitinib and 2 mg BID PD-0325901. Other continuous and intermittent administration regimens were investigated. The study is registered at ClinicalTrials.gov (NCT02039336).

**Results** We enrolled 38 patients, of whom 27 had CRC, eight NSCLC, and three patients had pancreatic cancer. The most common treatment-related adverse events were maculopapular and papulopustular rash (90%), diarrhea (87%) and nausea (59%). Dose-limiting toxicities occurred in seven patients and were grade 3 increased aspartate and alanine aminotransferase, fatigue, skin rash, dehydration, dyspnea, grade 2 neuropathy and the inability to take ≥75% of the assigned dose in the first four weeks of treatment due to grade 2 diarrhea and fatigue. The RP2D with continuous dacomitinib dosing was established at 15 mg dacomitinib QD plus 6 mg PD-0325901 (21 days on/7 days off). Intermittent dosing schedules are currently being explored. Significant pharmacokinetic drug-drug interactions were not observed. Tumor regression was seen in seven patients, of whom five had NSCLC, one CRC and one pancreatic cancer. In NSCLC patients, time on treatment was highest, with a median of 103 days.

**Conclusions** Dacomitinib can be combined safely with PD-0325901, albeit not at single agent full doses, with manageable toxicity. Signs or preferential activity in NSCLC were observed, which is why further recruitment is focused on NSCLC. Intermittent dosing schedules will be explored in an effort to enhance anti-tumor activity and to establish RP2D with an optimal efficacy to toxicity balance.

## INTRODUCTION

The Ras-Raf-MEK-ERK (MAPK) pathway plays a pivotal role in the regulation of cell proliferation, survival and differentiation. Persistent activation of this pathway is frequently observed in human cancers and is associated with high rates of cancer cell proliferation. Commonly, pathway activation occurs as a consequence of oncogenic gain-of-function mutations in Kirsten rat sarcoma viral oncogene homolog (*KRAS*). The KRAS protein stimulates multiple downstream effector pathways, which are activated in a growth factor-independent way in cancer cells expressing oncogenic *KRAS*[1–3]. The high frequency of *KRAS* mutations in human cancers (~20%)[4] with loss of treatment options makes targeting of these mutated proteins that lead to sustained pathway activation an attractive strategy for cancer therapy. The frequency of *KRAS* mutations is high in pancreatic cancer (90%), colorectal cancer (CRC) (45%) and non-small cell lung cancer (NSCLC) (35%)[4]. To date, therapeutic approaches developed to target and block KRAS directly have been unsuccessful. Small molecule inhibitors against the downstream effectors of KRAS, such as MEK, demonstrated only limited anti-tumor activity in *KRAS* mutated (*KRAS*m) cancers as well.[5–7]

Preclinical work from Sun and colleagues revealed that in *KRAS*m cancer cells, inhibition of MEK leads to feedback activation of upstream tyrosine kinase receptors, human epidermal growth factor receptor 2 (HER2) and 3 (HER3) in particular, causing intrinsic resistance through reactivation of the MAPK and phosphoinositide 3-kinase (PI3K) pathways[8]. Concurrent treatment with a MEK inhibitor and an inhibitor of multiple HER receptor subtypes (pan-HER inhibitor) completely suppressed this feedback activation and resulted in synergistic anti-tumor activity in *KRAS*m cells *in vitro* and in xenograft models[8]. As proof of concept was obtained in *KRAS*m CRC and NSCLC models, we hypothesized that the anti-tumor activity of this therapeutic approach is independent of tumor histology. The unmet medical need for new anticancer agents in *KRAS*m tumors and the high frequency of *KRAS* mutations provided rationale to investigate the combination of a MEK and pan-HER inhibitor in humans.

In this phase I dose-finding study, we investigated the combination of the potent irreversible ATP-competitive inhibitor (*in vitro* $IC_{50}$ values of 6.0 nM, 45.7 nM and 74 nM against the human catalytic domains of HER1, HER2 and HER4) of the HER kinase family dacomitinib with the highly specific non-ATP-competitive inhibitor of MEK1 and MEK2 PD-0325901, in patients with *KRAS*m CRC, NSCLC or pancreatic cancer. The primary study objective was to determine the recommended phase II dose (RP2D) and the most tolerable schedule of dacomitinib plus PD-0325901. Secondary objectives included characterizing safety and tolerability, exploring anti-tumor activity, and assessing the pharmacokinetic profiles of dacomitinib and PD-0325901 when given concomitantly. In this report, we provide an interim analysis of the ongoing clinical study.

## PATIENTS AND METHODS

### Patient population

This investigator-initiated, multi-center, open-label, phase I dose-escalation study enrolled patients at three sites in The Netherlands. Adult patients with histologically- or cytologically-confirmed advanced CRC, NSCLC or pancreatic cancer were enrolled on the basis of documented *KRAS* mutation in exon 2, 3 or 4, and *PIK3CA* wildtype status. *PIK3CA* wildtype was required to avoid treatment resistance via activation of signaling proteins downstream of PI3K. Eligibility criteria included: Eastern Cooperative Oncology Group (ECOG) performance status of <2, life expectancy of ≥3 months, measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1, adequate bone marrow (absolute neutrophil count ≥1.5 x $10^9$/L, platelets ≥100 x $10^9$/L, hemoglobin ≥6.0 mmol/L), hepatic (total bilirubin ≤1.5 x upper limit of normal [ULN], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 x ULN), and renal (serum creatinine ≤1.5 x ULN) functions. Radiotherapy, immunotherapy, chemotherapy or any treatment with investigational medication within four weeks prior to study treatment were not allowed, and patients with a history of other primary malignancies were excluded with the exception of patients who had been disease-free for ≥3 years or with completely resected non-melanoma skin cancer. Additional exclusion criteria included symptomatic or untreated leptomeningeal disease, symptomatic brain metastasis, history of interstitial lung disease or pneumonitis, history of retinal vein occlusion, and prior therapy containing targeted drug combinations known to interfere with EGFR, HER2, HER3, HER4 or MAPK- and PI3K-pathway components, including PI3K, AKT, mTOR, BRAF, MEK and ERK. The study was conducted in accordance with guidelines for Good Clinical Practice as defined by the International Conference on Harmonization. Regulatory authorities and the institutional review boards approved the study protocol and all amendments, and all patients gave written informed consent, per Declaration of Helsinki recommendations. The study was registered at ClinicalTrials.gov (NCT02039336). Pfizer Inc. funded this study and provided the investigational drugs dacomitinib and PD-0325901.

**Study design and procedures**

Patients were treated at varying dose-levels of orally administered dacomitinib and PD-0325901 in cycles of 28 days. The starting doses were based on previous data from single agent phase I studies with both compounds, taking into account the potential for synergistic toxicity. Dose-level one consisted of 30 mg dacomitinib once daily (QD) continuously, which is 67% of its single agent recommended dose, and 2 mg PD-0325901 twice daily (BID) administered on the first 21 days of each 28-day cycle, which is 25% of its single agent recommended dose. Subsequently, PD-0325901 was escalated according to a classical 3+3 design with fixed maximum escalation increments. Dose-escalation decisions were based on safety evaluation of all evaluable patients, performed after completion of the first treatment cycle. Patients were considered evaluable for the dose-determining set if at least one cycle of study treatment was completed, with the minimum safety evaluation and drug exposure (≥75% of the planned doses of dacomitinib and PD-0325901) or if dose-limiting toxicity (DLT) had occurred during the first cycle. If one out of three patients experienced a DLT, the number of patients treated at that dose-level was expanded to a maximum of six. Dose-escalation continued until a dose-level was reached at which no more than one out of six patients experienced DLT during the first 28 days of treatment, provided that the single agent recommended doses of both compounds were not exceeded. Upon assessment of the RP2D of the two-drug combination with continuously dosed dacomitinib, intermittent dacomitinib dosing was investigated to optimize drug exposure and to enable selection of the most tolerable administration regimen. Patients were to continue study treatment until disease progression, unacceptable toxicity, or investigator's/patient's decision to discontinue. Safety was monitored throughout the treatment by physical examination, laboratory assessments, electrocardiography, ophthalmic evaluation and collection of adverse events. Adverse events were recorded according to Common Terminology Criteria for Adverse Events version 4.0. DLT was defined as an adverse event or laboratory abnormality occurring within the first treatment cycle that meets at least one of the criteria described in supplementary table S1. Radiologic tumor measurements were performed using computed tomography (CT) scans at baseline and every six weeks throughout the study. After a protocol amendment, the frequency was changed to every eight weeks. Tumor response was evaluated according to RECIST version 1.1[9]. Patients were evaluable for anti-tumor activity if at least one follow-up radiologic evaluation was performed after the start of study treatment.

**Pharmacokinetic and pharmacodynamic analyses**

For pharmacokinetic analyses, serial blood samples were obtained from all patients prior to treatment administration on day one, and 1, 2, 3, 4, 6, 8, 12, 24, 72, and 144 hours after the first dose. On day one of cycle 2, blood samples were drawn before and 1, 2, 3, 4, 6, 8, 12 and 24 hours after administration. Plasma samples were assayed using a validated high performance liquid chromatography tandem mass spectrometry method (HPLC-MS/MS). Briefly, dacomitinib and PD-0325901 were extracted from plasma by protein precipitation with a mixture of acetonitrile/methanol (1:1 v/v). Compounds were chromatographically separated using a Waters Xbridge BEH Phenyl column (50 x 2.1 mm ID, 5 µm particle size) and detection was performed using an API4000 tandem mass spectrometer equipped with a turbo ion spray interface, operating in the positive ion mode. Transitions from m/z 480 to 329 and m/z 489 to 255 were monitored for the detection of dacomitinib and PD-0325901, respectively. Stable labelled internal standards were used for the quantification. The lower and upper limits of quantification were respectively 0.5 and 50 ng/mL for dacomitinib, and 5 and 500 ng/mL for PD-0325901. Pharmacokinetic parameters were calculated in R using an in-house developed package for non-compartmental pharmacokinetic analyses (version 1.3)[10].

During the study, the protocol was amended to allow incorporation of tumor biopsies for pharmacodynamic analyses. Biopsies were taken before treatment, in the second week of treatment and upon treatment discontinuation. Phosphorylated (p) ERK and ribosomal pS6 (pS6-r) levels were assessed by validated immunohistochemistry (IHC) staining methods and semi-quantitative H-scores (percentage of positive cells (0–100) multiplied by staining intensity (0–3)) were assessed by an independent pathologist who was blinded for sample identification. Tumor biopsy samples were fixed in formalin for 16–24 hours and embedded in paraffin subsequently. Immunohistochemistry of formalin-fixed paraffin-embedded tumor samples was performed on a BenchMark Ultra autostainer (Ventana Medical Systems). Briefly, paraffin sections were cut at 3 µm, heated at 75°C for 28 minutes and deparaffinised in the instrument with EZ prep solution (Ventana Medical Systems). Heat-induced antigen retrieval was carried out using Cell Conditioning 1 (CC1, Ventana Medical Systems) at 95$^0$C for 32 and 64 minutes, for pS6-r and pERK1/2, respectively. pS6-r was detected using clone D68F8 (1:1000 dilution, 32 minutes at room temperature, Cell Signalling) and phospho-p44/42 MAPK (pERK1/2) (Thr202/Tyr204) using clone D13.14.4E (1:400 dilution, 1 hour at room temperature, Cell Signalling). pERK was detected using the UltraView Universal DAB Detection Kit (Ventana Medical Systems), while detection of pS6-r was performed using the OptiView DAB Detection Kit (Ventana Medical Systems). Slides were counterstained with hematoxylin.

**Statistical analysis**
Pharmacokinetics, pharmacodynamics, safety and tumor response data were reported descriptively. The statistical significance of pERK/pS6-r modulation during treatment was calculated using a paired students' T-test.

## RESULTS

**Patient disposition and characteristics**
In total, 38 patients were enrolled onto this study between April 2014 and the data cut-off on 15 May 2017, 27 patients (71%) with CRC, eight with NSCLC (21%) and three (8%) with pancreatic cancer. The majority of patients had *KRAS* exon 2 mutations and were pretreated with at least two prior lines of antineoplastic therapy for advanced disease (table 1). One patient did not have any antineoplastic therapy which was allowed per protocol. Thirty-three patients were evaluable for dose-determination; three patients were considered not evaluable due to clinical deterioration, patient refusal and mistakenly administration of the wrong dose and two patients did not complete the DLT period at time of data cut-off. At data cut-off, two patients were ongoing, and 36 patients had discontinued treatment due to progressive disease (n=27), adverse events (n=5), clinical deterioration/lack of benefit (n=3), or patient refusal (n=1).

**Table 1.** Patient and disease characteristics at baseline. *Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; KRAS, Kirsten rat sarcoma viral oncogene homolog.*

| | Patients (n = 38) | |
|---|---|---|
| Sex, n (%) | | |
| Female | 21 | (55%) |
| Male | 17 | (45%) |
| Age, median (range), years | 62 | (43–81) |
| Tumor types, n (%) | | |
| Colorectal | 27 | (71%) |
| Non-small cell lung | 8 | (21%) |
| Pancreatic | 3 | (8%) |
| ECOG PS, n (%) | | |
| 0 | 14 | (37%) |
| 1 | 24 | (63%) |
| Number of prior lines of therapy, n (%) | | |
| 0 | 1 | (3%) |
| 1 | 7 | (18%) |
| 2 | 11 | (29%) |
| ≥ 3 | 19 | (50%) |
| KRAS mutation, n (%) | | |
| Exon 2 | 34 | (90%) |
| Exon 3 | 2 | (5%) |
| Exon 4 | 2 | (5%) |

**Dose finding**
At the first dose-level consisting of 30 mg QD dacomitinib plus 2 mg BID PD-0325901 (21 days on/7 days off), three out of six patients experienced DLTs, being grade 3 increased AST/ALT, grade 3 fatigue, and inability to receive at least 75% of the planned dose due to grade 2 fatigue and diarrhea. Therefore, we decided to continue with a reduced dacomitinib dose of 15 mg in a continuous dosing schedule to allow for escalation of PD-0325901. In the subsequent dose-levels with continuous administration of dacomitinib, DLTs were reported in two out of 27 patients; grade 3 AST/ALT increase (dose-level 2) and grade 3 skin rash (dose-level 5), respectively (figure 1). Although the formal RP2D was not reached, the escalation of PD-0325901 was halted in view of the increasing number of multiple grade 2 adverse events (e.g. diarrhea, nausea, fatigue) beyond the DLT window of 28 days, together with the emergence of ocular toxicity at dose-level 5 (including retinopathy grade 1, retinal detachment grade 1 and grade 2 and dry eyes grade 1). The latter with knowing the potential of more severe ocular toxicity at higher PD-0325901 doses[11,12]. Consequently, the established maximum dose-level with continuous dacomitinib dosing consisted of 15 mg dacomitinib QD plus 6 mg PD-0325901 BID. Subsequently, intermittent regimens were initiated with the aim of optimizing exposure and tolerability.

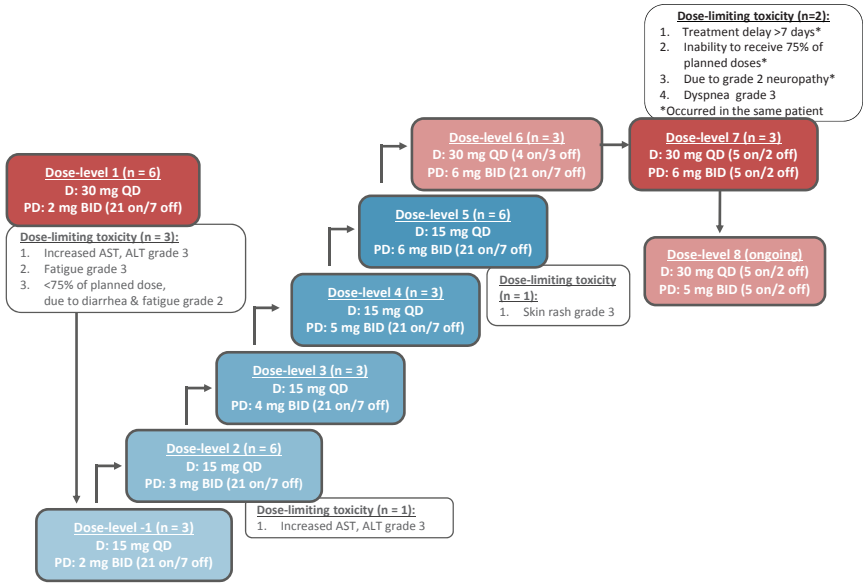


**Figure 1.** Dose-escalation cohorts and dose-limiting toxicities. *Abbreviations: D, dacomitinib; PD, PD-0325901; QD, once daily; BID, twice daily; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; AST, aspartate aminotransferase; ALT, alanine aminotransferase; n, number of patients.*

A slight increase in exposure to dacomitinib was intended with dose-level 6 consisting of 30 mg dacomitinib QD 4 days on/3 days off for 28 days, and PD-0325901 6 mg BID for 21 days. No DLTs were observed at this dose-level, which allowed further escalation of dacomitinib to 30 mg QD 5 days on/2 days off. In view of patient convenience, it was decided to use a 5 days on/2 days off regimen for both agents which should increase the exposure of dacomitinib with the same dose. Out of three patients, two experienced DLTs consisting of grade 2 neuropathy leading to treatment delay of >7 days and inability to receive 75% of the planned doses in one patient, and dyspnea grade 3 in the other patient. This warranted dose de-escalation. Because a 5 days on/2 days off regimen was still considered preferential, this regimen was maintained and PD-0325901 was de-escalated to 5 mg PD-0325901 BID combined with 30 mg dacomitinib QD in dose-level 8. This dose-level was ongoing at the time of data-cut-off.

## Safety

Study treatment-related adverse events were reported in all patients, with the most common being maculopapular and papulopustular rash (90%), diarrhea (87%), nausea (59%), vomiting (41%) and fatigue (33%) (table 2). Supportive care, including minocycline and cetomacrogole cream or class I corticosteroid cream were sufficient to manage skin rash, with the exception of one patient in dose-level 5 who had to discontinue treatment due to dose-limiting skin rash. The most frequent grade 3 events were diarrhea (23%), nausea (10%), and fatigue (8%). Diarrhea caused treatment interruption in five patients, nausea in three patients and fatigue caused discontinuation in two patients. In all other cases, supportive care was sufficient to decrease the severity to grade 1 or less. Eye toxicities included grade 1 retinopathy, dry eyes grade 1, watering eyes grade 1, retinopathy grade 1 and retinal detachment which occurred in four patients in dose-levels 1, 5 and 6. All patients could continue study treatment without further progression of ocular toxicity. Cases of retinal vein occlusion were not observed in this study.

## Pharmacokinetic analysis

Pharmacokinetic parameters after the first dose and at steady-state are summarized in table 3. PD-0325901 and dacomitinib exposure increased approximately dose-proportionally with moderate and high inter-patient variability, respectively (table 3, figure 2). The half-life of dacomitinib could not be accurately calculated by non-compartmental analysis, due to its long terminal half-life. The difficult estimation and the high variability is known from previous studies and is also reflected in our results[13]. The mean dacomitinib peak plasma concentration ($C_{max}$) and area under the plasma concentration-time curve from time 0 to 24 hours ($AUC_{0\text{-}24h}$) increased approximately three-fold after multiple dosing. A slight increase in AUC and $C_{max}$ was also observed for PD-0325901 after multiple doses. Figure 2 shows the individual plasma-concentration time curves with the preclinical target levels for both drugs, which will be discussed in the discussion section.

**Table 2.** Treatment-related adverse events, occurring in >10% of patients.
*^Some patients experienced one or more skin toxicities, only one was counted for the combined group of any toxicity * includes neurosensory detachment, blurred vision, retinopathy, cataract and dry eyes.*
*Abbreviations: QD, once daily; BID, twice daily; ALT/AST, alanine/aspartate aminotransferase; 4/3, 4 days on / 3 days off; 5/2, 5 days on/2 days off; CPK, creatine phosphokinase; PPE, palmar plantar dysthesia syndrome.*

| | Dose-level -1 (n = 4) | | Dose-level 1 (n = 6) | | Dose-level 2 (n = 6) | | Dose-level 3 (n = 3) | | Dose-level 4 (n = 3) | | Dose-level 5 (n = 8) | | Dose-level 6 (n = 3) | | Dose-level 7 (n = 3) | | Dose-level 8 (n = 3) | | **Total (n = 39)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dacomitinib QD<br>PD-0325901 BID | 15 mg<br>2 mg | | 30 mg<br>2 mg | | 15 mg<br>3 mg | | 15 mg<br>4 mg | | 15 mg<br>5 mg | | 15 mg<br>6 mg | | 30 mg (4/3)<br>6 mg | | 30 mg (5/2)<br>6 mg (5/2) | | 30 mg (5/2)<br>5 mg (5/2) | | **All dose-levels** |
| **Adverse Event, n (%)** | **Gr 1/2** | **Gr 3** | **Gr 1/2** | **Gr 3** | **Gr 1/2** | **Gr 3** | **Gr 1/2** | **Gr 3** | **Gr 1/2** | **Gr 3** | **Gr 1/2** | **Gr3** | **Gr 1/2** | **Gr 3** | **Gr 1/2** | **Gr 3** | **Gr 1/2** | **Gr 3** | |
| Any skin toxicity^ | 3 (8%) | | 5 (5%) | 1 (3%) | 6 (15%) | | 3 (8%) | | 3 (8%) | | 5 (13%) | 1 (3%) | 3 (8%) | | 3 (8%) | | 2 (5%) | | **35 (90%)** |
| *Rash* | 3 (8%) | | 5 (5%) | 1 (3%) | 6 (15%) | | 3 (8%) | | 3 (8%) | | 4 (10%) | 1 (3%) | 3 (8%) | | 2 (5%) | | 2 (5%) | | **33 (85%)** |
| *Dry skin* | | | 3 (8%) | | 1 (3%) | | | | | | 2 (5%) | | 1 (3%) | | 1 (3%) | | 1 (3%) | | **9 (23%)** |
| *Skin fissures* | | | 2 (5%) | | | | 2 (5%) | | | | | | | | | | | | **4 (10%)** |
| *PPE* | 1 (3%) | | | | 1 (3%) | | 1 (3%) | | 1 (3%) | | 2 (5%) | | | | | | | | **6 (15%)** |
| Diarrhea | 4 (10%) | | 6 (15%) | 2 (5%) | 4 (10%) | | 1 (3%) | 1 (3%) | 2 (5%) | | 4 (10%) | 3 (8%) | 1 (3%) | 1 (3%) | 3 (8%) | | 1 (3%) | 1 (3%) | **34 (87%)** |
| Nausea | 4 (10%) | | 2 (5%) | 1 (3%) | 1 (3%) | 1 (3%) | 2 (5%) | | 2 (5%) | 1 (3%) | 5 (13%) | 1 (3%) | 2 (5%) | | | | 1 (3%) | | **23 (59%)** |
| Vomiting | 3 (8%) | | 2 (5%) | | 1 (3%) | 1 (3%) | 2 (5%) | | 1 (3%) | | 5 (13%) | 1 (3%) | | | | | | | **16 (41%)** |
| Fatigue | 1 (3%) | | 3 (8%) | 1 (3%) | 1 (3%) | 1 (3%) | 1 (3%) | | | | 3 (8%) | | | 1 (3%) | 1 (3%) | | | | **13 (33%)** |
| Anorexia | 1 (3%) | | 3 (8%) | | 1 (3%) | | 1 (3%) | | 1 (3%) | | 3 (8%) | | | | | | 1 (3%) | | **11 (28%)** |
| CPK increased | | | 1 (3%) | 1 (3%) | 2 (5%) | | 1 (3%) | | 1 (3%) | | 2 (5%) | | 2 (5%) | | | | | | **10 (26%)** |
| ALT/AST increased | | | 3 (8%) | 1 (3%) | 1 (3%) | 1 (3%) | | | 1 (3%) | | 1 (3%) | | 1 (3%) | | | | | | **9 (23%)** |
| Mucositis | 2 (5%) | | 1(3%) | | 1 (3%) | | 1 (3%) | | | | 2 (5%) | | 2 (5%) | | | | | | **9 (23%)** |
| Eye toxicity* | | | 3 (8%) | | | | | | 1 (3%) | | 2 (5%) | | 2 (5%) | | | | | | **8 (25%)** |
| Alopecia | | | 3 (8%) | | 1 (3%) | | 1 (3%) | | | | 1 (3%) | | | | | | | | **5 (13%)** |
| Dry mouth | | | 2 (5%) | | 1 (3%) | | | | 1 (3%) | | 1 (3%) | | | | 1 (3%) | | | | **5 (13%)** |

**Anti-tumor activity**

Thirty-three patients were evaluable for anti-tumor activity; four patients did not reach the first radiological evaluation due to clinical deterioration (n=1), adverse events (n=1), patient refusal (n=1) or insufficient treatment (n=1) and for one patient, tumor scans were not available at the time of data cut-off. Out of the evaluable patients, 18 achieved stable disease and 15 had progressive disease on their first evaluation scan (figure 3). Tumor regression was seen in seven patients (18%) treated at dose-levels 1, 3, 5, 6 and 8. Out of the six evaluable patients with NSCLC, five achieved tumor regression within the limits of stable disease according to RECIST v1.1 criteria and one had no change in target lesion volume as best response. The overall median treatment duration was 53 days (range 3–469). Patients with NSCLC achieved the longest median treatment duration, 103 days (range 14–239), versus 81 days (42–96) for patients with pancreatic cancer and 43 days (range 3–469) for patients with CRC. Median treatment duration was the longest in the dose-levels that contained 30 mg dacomitinib. In dose-level 1 with 30 mg dacomitinib and 2 mg PD-0325901, treatment duration was the longest (239 days, range 42–469), followed by dose-level 8 (dacomitinib 30 mg and PD-0325901 5 mg, both 5 days on/2 days off [88 days, range 60–116]) and 6 (dacomitinib 30 mg 4 days on/3 days off and PD-0325901 6 mg [79 days, range 49–96]) (figure 4).

**Pharmacodynamic analyses**

Tumor biopsies were taken from five patients at baseline and from two patients also on-treatment (figure 5). In the two patients from whom a paired biopsy was available, pERK was increased whereas pS6 was slightly decreased in one patient and increased in the other patient. Instead of increases, reductions would be expected based on results from other studies described in this thesis (chapter 2.1 and 2.3). Importantly, for one of the two patients, the biopsy was taken after four days of study treatment interruption, which may explain the lack of pERK modulation. In the other patient, the formalin-fixation of the baseline biopsy was delayed whereas direct fixation was desired. This delay might have caused degradation of phosphorylated proteins. These deviations will be discussed further in the next section.

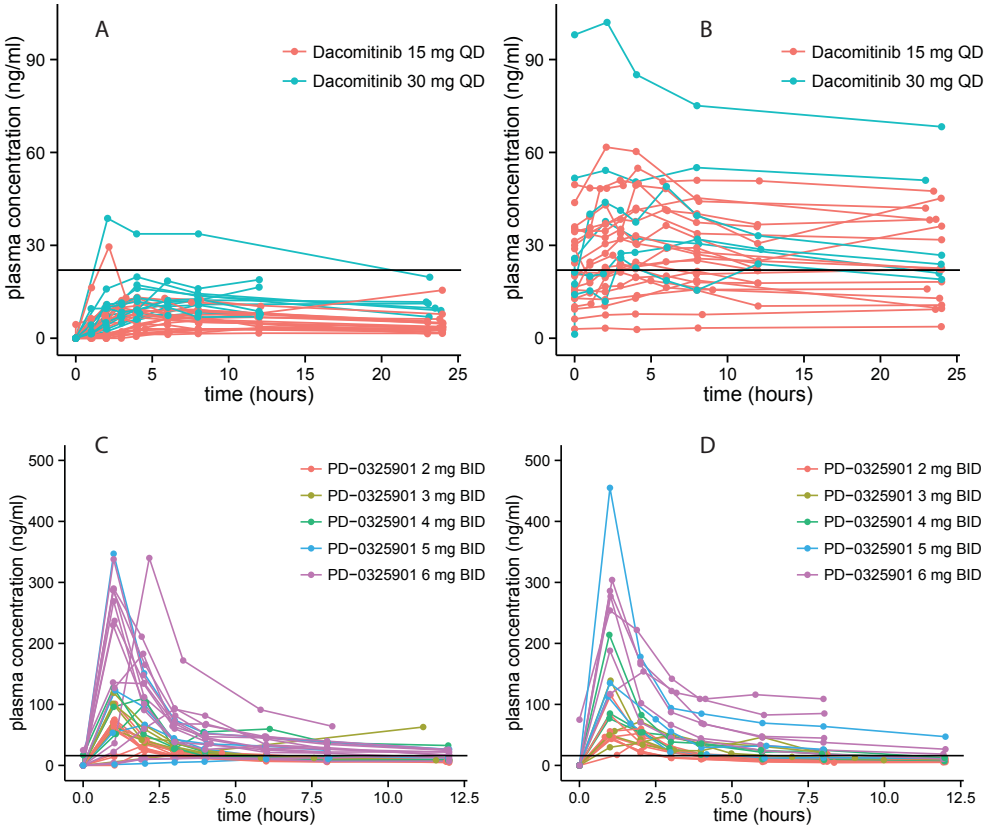


**Figure 2.** Plasma concentration time curves of dacomitinib and PD-0325901 on cycle 1 day 1 (A, C) and cycle 2 day 1 (B, D) per dose. The black line represents the target levels of 16.5 ng/mL for PD-0325901 and of 22 ng/mL for dacomitinib.

**Table 3.** Pharmacokinetic parameters of dacomitinib and PD-0325901, at baseline and steady-state, per dose-level. Data from patients with dacomitinib dose interruptions less than 7 days prior to cycle 2 day 1 were excluded in this analysis. *Abbreviations: $C_{max}$, peak plasma concentration; $T_{max}$, time of maximum plasma concentration observed; $AUC_{0-24h}$, area under the plasma concentration-time curve from time zero to 24 hours; $T_{1/2}$, elimination half-life; N/C, could not be calculated; 4/3, 4 days on/3 days off; 5/2, 5 days on/2 days off.*

| **Dose-level** | **1** | **-1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Dacomitinib QD* | 30 mg | 15 mg | 15 mg | 15 mg | 15 mg | 15 mg | 30 mg (4/3) | 30 mg (5/2) | 30 mg (5/2) | | |
| *PD-0325901 BID* | 2 mg | 2 mg | 3 mg | 4 mg | 5 mg | 6 mg | 6 mg | 6 mg (5/2) | 5 mg (5/2) | | |
| **Dacomitinib** | *Cycle 1 Day 1* | | | | | | | | | ***All 15 mg doses*** | ***All 30 mg doses*** |
| *Mean (CV%)* | n = 6 | n = 3 | n = 6 | n = 2 | n = 3 | n = 8 | n = 2 | n = 3 | n = 1 | n = 28 | n = 12 |
| $C_{max}$ (ng/mL) | 19.2 (52) | 7.5 (53) | 6.7 (61) | 2.5 (48) | 8.5 (11) | 11.4 (74) | 10.4 (30) | 19.1 (38) | 10.7 | 10.7 (77) | 17.0 (50) |
| $T_{max}$ (h) | 7.5 | 4.9 | 8.7 | 12 | 4.7 | 7.9 | 8.0 | 13.3 | 8.0 | 7.6 | 9.1 |
| $AUC_{0-24h}$ (ng*h/mL) | 322 (51) | 121 (50) | 112 (57) | 44.6 (36) | 137 (17) | 136 (60) | 188 (20) | 277 (58) | 174 | 162 (76) | 276 (52) |
| $T_{1/2}$ (h) | N/C | 57 | N/C | N/C | 32 | 61 | 44 | N/C | 32 | N/C | 9.4 |
| **Dacomitinib** | *Cycle 2 Day 1* | | | | | | | | | | |
| *Mean (CV%)* | n = 5 | n = 2 | n = 5 | n = 2 | n = 4 | n = 5 | n = 1 | n = 1 | | n = 23 | n = 12 |
| $C_{max}$ (ng/mL) | 55.0 (56) | 22.9 (118) | 26.0 (44) | 25.7 (18) | 37.5 (41) | 37.3 (33) | 32 | 25.9 | | 36.4 (56) | 47.6 (60) |
| $T_{max}$ (h) | 4.4 | 14 | 9.6 | 2 | 4.8 | 4.4 | 8.0 | 3.0 | | 6.2 | 4.8 |
| $AUC_{0-24h}$ (ng*h/mL) | 1031 (56) | 452 (116) | 549 (45) | 262 (11) | 747 (38) | 747 (43) | 647 | 482 | | 697 (58) | 897 (59) |
| **PD-0325901** | *Cycle 1 Day 1* | | | | | | | | | ***All 6 mg doses*** | |
| *Mean (CV%)* | n = 6 | n = 3 | n = 5 | n = 3 | n = 3 | n = 8 | n = 2 | n = 3 | n = 1 | n = 13 | |
| $C_{max}$ (ng/mL) | 68.7 (32) | 43.7 (59) | 88.0 (29) | 113.3 (11) | 179 (83) | 262 (30) | 160 (21) | 262 (30) | 262 | 209.1 (53) | |
| $T_{max}$ (h) | 1.2 | 1.3 | 1.0 | 1.7 | 1.4 | 1.4 | 1.5 | 1.4 | 1.4 | 1.3 | |
| $AUC_{0-12h}$ (ng*h/mL) | 188 (20) | 160 (20) | 269 (16) | 345 (68) | 552 (55) | 776 (30) | 468 (60) | 279 (101) | 86.3 | 614.3 (52) | |
| $T_{1/2}$ (h) | 9.7 | 7.1 | 7.9 | 8.8 | 7.6 | 6.0 | 13.5 | 4.4 | 12.7 | 7.8 | |
| **PD-0325901** | *Cycle 2 Day 1* | | | | | | | | | | |
| *Mean (CV%)* | n = 6 | n = 3 | n = 5 | n = 2 | n = 4 | n = 5 | n = 1 | | | n = 6 | |
| $C_{max}$ (ng/mL) | 58.3 (28) | 47.7 (38) | 73.8 (51) | 125 (61) | 229 (68) | 242 (27) | 254 | | | 244 (24) | |
| $T_{max}$ (h) | 1.5 | 1.1 | 4.2 | 1 | 1 | 1.2 | 1 | | | 1.2 | |
| $AUC_{0-12h}$ (ng*h/mL) | 210 (15) | 145 (29) | 279 (21) | 376 (33) | 638 (65) | 855 (35) | 1341 | | | 936 (36) | |
| $T_{1/2}$ (h) | 5.0 | 9.8 | 5.7 | 7.7 | 9.9 | 7.5 | 8.4 | | | 7.6 | |

## DISCUSSION

In this clinical study we investigated a combination of the MEK inhibitor PD-0325901 with the pan-HER inhibitor dacomitinib. For the first time, we demonstrated that dacomitinib could be combined safely with PD-0325901, although not at full single agent doses. In a previous phase I dose-escalation study, PD-0325901 doses up to 20 mg BID in a continuous dosing schedule, 30 mg BID in a 21 days on/7 days off schedule, and 10 mg in a 5 days on/2 days off schedule were investigated. Although formal RP2Ds were established at 15 mg BID and 10 mg BID on continuous and 5 days on/2 days off schedules, respectively, occurrence of ocular toxicity, retinal vein occlusion in particular, prevented a clear definition of a RP2D[11]. As dacomitinib shows potential overlapping toxicity with PD-0325901, starting doses for both agents were 20-50% of their monotherapy doses, being 2 mg PD-0325901 BID in a 21 days on/7 days off schedule and 30 mg dacomitinib QD. Although relatively low, these doses demonstrated target engagement and clinical activity in their respective single agent studies[11,13]. Nevertheless, the initial dose-level was already intolerable as indicated by DLTs in three out of six patients. Given the relatively low dose of PD-0325901 in relation to its single agent maximum-tolerated dose, toxicity was likely to be associated with dacomitinib in particular. Therefore, the dacomitinib dose was reduced to enable dose-escalation of PD-0325901, as we hypothesized that robust MEK inhibition was necessary to block the KRAS-activated MAPK pathway before tumor cells activate their escape mechanism through upstream tyrosine kinase receptors[8]. Because ocular toxicity, i.e. asymptomatic central serous retinopathy, emerged at the 5 mg and 6 mg dose-levels, we halted dose escalation at 6 mg and established the RP2D with continuous dacomitinib dosing at 15 mg dacomitinib QD plus 6 mg PD-0325901 BID 21 days on/7 days off. At doses of 5 and 6 mg, the plasma concentration of PD-0325901 exceeded the target level (16.5 ng/mL), consistent with target inhibition based on xenograft mouse models[14], during the entire dose interval (figure 2C). However, at 15 mg dacomitinib doses, the plasma concentration did not exceed the preclinical target of 22 ng/ml, which is the $IC_{50}$ for HER2/HER3 inhibition (unpublished data), for a substantial number of patients (figure 2A).

Therefore, after determination of the RP2D with continuous dacomitinib dosing, intermittent dosing schedules were initiated in an effort to optimize exposure and preserve tolerability. Dacomitinib 30 mg QD 4 days on/3 days off combined with PD-0325901 6 mg BID 21 days on/7 days off was safe and well tolerated and further exploration of a 5 days on/2 days on regimen is ongoing. Unfortunately, the biopsies taken from patients treated at the latest dose-levels could not confirm target engagement in terms of pERK and pS6 modulation. This is mainly explained by the fact that data was available from two patients only, and for both patients tumor biopsies were not collected according to the protocol. Target engagement was clearly confirmed in two other studies with MEK and pan-HER inhibitors, described in chapter 2.1 and 2.3.

Pharmacokinetic parameters of both agents were in line with previously reported single agent data. Our data show no signs of pharmacokinetic interactions between the two agents[11,13]. The low number of patients in each dose-level, together with the high interpatient variability in pharmacokinetic parameters may explain the low dacomitinib exposure in dose-level 6 and 8 relative to dose-level 1 and 7 (table 3).

Patients with metastatic *KRAS*m tumors represent a population with high unmet medical needs. Multiple strategies to target KRAS have been explored, including farnesyltransferase inhibitors, small molecules interfering with the prenyl-binding protein PDEδ-KRAS interaction, and small molecules targeting downstream effectors of KRAS, e.g. RAF, MEK, or PI3K. However, none of these approaches has been successful[6,15,16]. Since all these strategies rely on targeting a single protein or pathway, rapid onset of resistance due to tumor escape exploiting alternative pathways is to be expected[17]. Therefore, combination strategies may have a more sustained anti-tumor effect. Previously, van Geel *et al.* demonstrated clinical proof of concept for combining BRAF and EGFR inhibition in patients with *BRAF*m CRC[18], based on a synthetic lethality drug screen[19]. Similarly, in *KRAS*m cells, inhibition of MEK was found to synergize with HER2 and HER3 inhibition in an identical screen to identify synthetic lethal interactions[8]. However, in contrast to these preclinical observations, the preliminary clinical activity with dacomitinib plus PD-0325901 in *KRAS*m tumors was relatively low. Although dose-escalation is ongoing, toxicity restricted combining full single agent doses, which potentially limits clinical anti-tumor activity due to lower exposure. It remains to be established whether any of the currently explored dose levels in intermittent schedules will do better clinically.

Another explanation for the limited anti-tumor activity thus far may lie in the extensive inter-pathway connections of the KRAS protein. Although we excluded patients with concurrent *KRAS* and *PIK3CA* mutations, activation of the PI3K pathway may as well be triggered by mutated *KRAS* directly, particularly in the presence of downstream MEK inhibition[20]. Additionally, reactivation of the MAPK pathway may occur as well, analogous to the observation with *BRAF* inhibition in *BRAF*m CRC cells[21], especially when upstream receptors are not

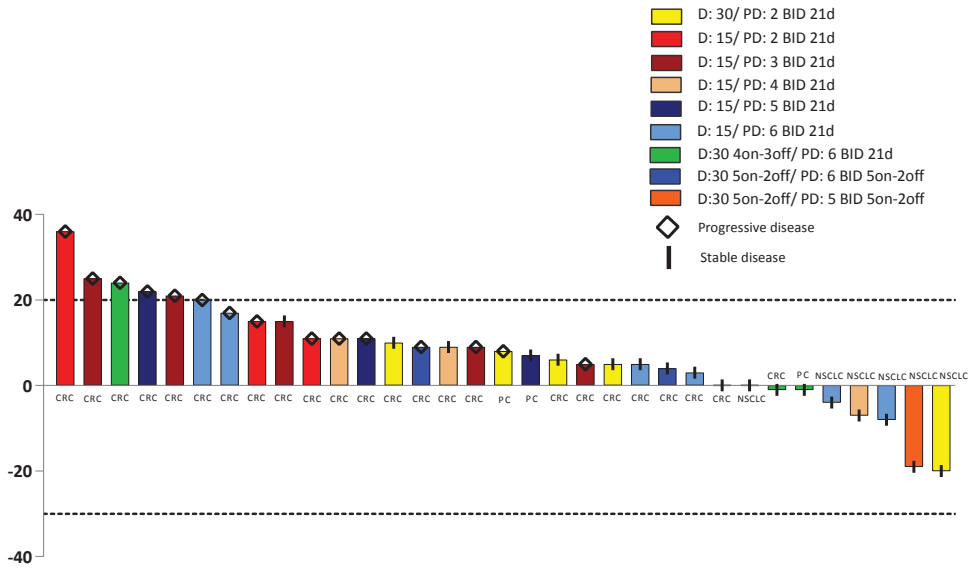


**Figure 3.** Maximum percentage change in sum of target lesion size from baseline, including responses assessed by RECIST, by dose-level.
*Abbreviations: D, dacomitinib; PD, PD-0325901; QD, once daily; BID, twice daily; CRC, colorectal cancer; NSCLC, non-small cell lung cancer.*

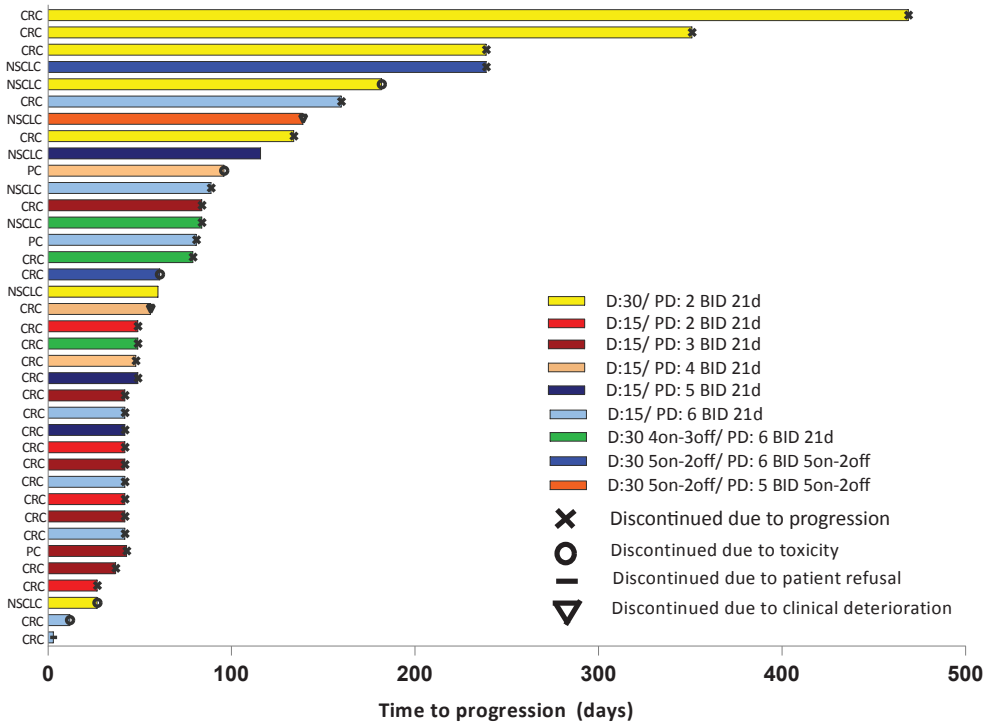


**Figure 4.** Swimmer plot of treatment duration, by dose-level. Symbols at the end of each bar represent the reason for end of treatment for each individual patient.
*Abbreviations: D, dacomitinib; PD, PD-0325901; QD, once daily; BID, twice daily; CRC, colorectal cancer; NSCLC, non-small cell lung cancer.*

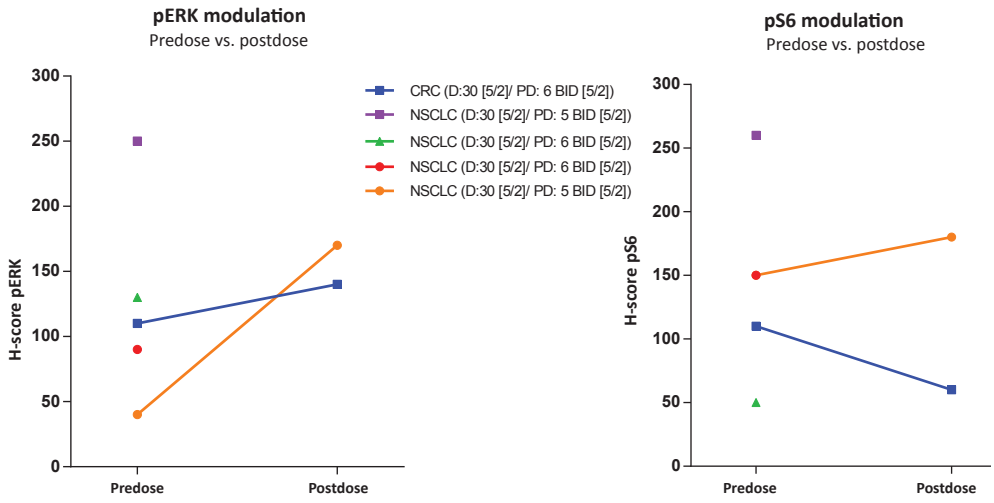


**Figure 5.** Pharmacodynamic effects of dacomitinib and PD-0325901. The pERK and p-S6r intensity scores (H-scores) of tumor biopsies at baseline and on-treatment (day 15) are presented as determined by immunohistochemistry staining.
*Abbreviations: D, dacomitinib; PD, PD-0325901; BID, twice daily; CRC, colorectal cancer; NSCLC, non-small cell lung cancer.*

adequately inhibited. Indeed, although this concerns a small cohort, patients treated with doses of 30 mg dacomitinib had disease stabilization for a longer period of time compared to patients on dose-levels containing 15 mg dacomitinib (figure 4).

Interestingly, seven out of eight patients (88%) with NSCLC achieved tumor regressions, compared to one out of 24 patients (4%) with CRC (figure 3). In addition, the median treatment duration in patients with NSCLC (103 days) was longer than that of CRC patients (43 days), suggesting a difference in sensitivity to study treatment between these histological subtypes (figure 4). This finding was also reflected in the results of two separate studies. Höchster *et al.* showed that adding a MEK inhibitor to second line irinotecan therapy in patients with *KRAS*m CRC did not result in clinical benefit[22]. However, patients with *KRAS*m NSCLC had an improved response rate by the addition of the MEK inhibitor selumetinib to second line treatment with docetaxel as reported by Jänne *et al.,* although no significant effect on progression-free survival and overall survival was observed[23]. In addition, the same trend towards preferential activity in NSCLC was observed in the lapatinib-trametinib combination as described in chapter 2.3. To allow confirmation of this observation, the protocol was amended in December 2016 to restrict inclusion to patients with NSCLC only. To explain differences in sensitivity between tumor types, several biomarkers will be explored. As HER3 protein expression levels seemed to have predictive potential in preclinical studies[8], evaluation of HER3 and heregulin expression in patient samples is planned. Also, elevated Bcl-xL levels have been reported to cause resistance in *KRAS* mutant tumors treated with MEK inhibitors which may explain differences in sensitivity as well[24]. Thirdly, the ratio *KRAS* mutant to *KRAS* wildtype, *KRAS* copy numbers and *KRAS* expression levels could be of interest, given their strong involvement in MAPK-pathway activation[25]. To optimize the treatment for patients with *KRAS*m tumors, various treatment strategies have emerged from synthetic lethal screens and new agents have been developed[26,27]. Currently, researchers are working towards designing small molecules tailored to the surface of KRAS proteins, while others develop small molecules that target KRAS in a mutation-specific manner[28–30] or focus on deploying small interfering RNA to target KRAS[31–33]. Time will tell whether any of these strategies will result in clinical benefit for this patient population.

In conclusion, the combination of dacomitinib plus PD-0325901 was tolerable albeit only at doses lower than the recommended single agent doses. So far, only modest clinical activity was observed and toxicity prevented high continuous dosing of dacomitinib. The ongoing study will focus on confirming the signals of activity in NSCLC patients and on determining a dose and regimen that has an optimal efficacy to toxicity ratio.

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## SUPPLEMENT

**Table S1.** Criteria for defining dose-limiting toxicities.
*Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ULN, upper limit of normal; LLN, lower limit of normal; DLT, dose limiting toxicity.*

| Toxicity | DLT definition |
|---|---|
| Hematologic | • Grade 4 neutropenia for ≥5 days<br>• Grade ≥3 febrile neutropenia<br>• Grade 4 anemia<br>• Grade 4 thrombocytopenia |
| Non-hematologic | • AST >5X ULN OR, ALT >3X ULN AND bilirubin >2X ULN (after exclusion of disease progression and/or bile duct obstruction)<br>• Grade ≥4 rash, hand-foot syndrome or photosensitivity<br>• Grade 3 rash, hand-foot syndrome or photosensitivity for >7 days despite adequate supportive treatment.<br>• Grade ≥3 nausea, vomiting or diarrhea in the presence of maximal supportive care<br>• Grade ≥2 peripheral sensory or motor neuropathy<br>• Grade ≥3 clinically significant non-hematologic toxicity other than those listed above, with the following exceptions:<br>o Electrolyte disturbances that respond to correction within 24 hours<br>o Grade 3 hypertension that is adequately controlled by the addition of up to 2 additional antihypertensive medications<br>o Grade 3 pyrexia that does not result in study discontinuation |
| Cardiac | • Ejection fraction < lower limit of normal with an absolute decrease of >10% from baseline with confirmation within 14 days |
| Other | • Inability to receive ≥75% of scheduled doses in treatment period due to toxicity related to study treatment<br>• Treatment delay of >7 days due to study treatment-related toxicity<br>• Grade ≥2 toxicity that occurs beyond 28 days which in the judgment of the investigator is a DLT |

## Chapter 2.3

**Phase I study of lapatinib plus trametinib in patients with *KRAS*-mutation positive colorectal, non-small cell lung and pancreatic cancer**

*Interim analysis*

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**ABSTRACT**

**Background** Mutations in the *KRAS* oncogene are amongst the most powerful cancer drivers, causing sustained signaling through the mitogen-activated protein kinase (MAPK) pathway to stimulate uncontrolled cell growth. Until now, efforts to target KRAS directly have been unsuccessful. Pharmacological inhibition of its downstream effectors provided disappointing results in the clinic as well. Preclinical research revealed that KRAS mutated (*KRAS*m) cells are intrinsically resistant to MEK inhibitors due to feedback activation of upstream growth receptors that reactivate the MAPK and phosphoinositide 3-kinase (PI3K) pathway. Concurrent inhibition of MEK and the human epidermal growth factor receptor family members EGFR and HER2 resulted in synergistic anti-tumor activity, with complete inhibition of tumor growth *in vitro* and *in vivo*.

**Methods** We undertook an investigator-initiated, single-center, phase I dose-escalation study to assess the safety, tolerability and anti-tumor activity of the MEK inhibitor trametinib combined with the dual EGFR/HER2 inhibitor lapatinib in patients with advanced *KRAS*m and *PIK3CA* wildtype colorectal cancer (CRC), non-small cell lung cancer (NSCLC) and pancreatic cancer. Patients received escalating doses of continuous or intermittent once daily (QD) orally administered lapatinib and trametinib, starting at 750 mg and 1 mg continuously, respectively. The primary objective of this study was to determine the recommended phase 2 dose and most tolerable schedule of this combination. The study was registered at ClinicalTrials.gov (NCT02230553).

**Results** At data cut off, 27 patients were enrolled across five different dose-levels; 16 patients with CRC, eight with NSCLC, and three patients with pancreatic cancer. Dose-limiting adverse events were reported in seven patients at four different dose-levels; grade 3 diarrhea (n=3), rash (n=2), nausea (n=1), and aspartate aminotransferase elevation (n=1) which resulted in the inability to receive 75% of the planned doses (n=2) or treatment delay (n=2). The most frequently reported adverse events of any grade were skin rash (89%), diarrhea (81%) and fatigue (56%). The maximum tolerated dose with continuous dosing was established at 750 mg lapatinib QD plus 1 mg trametinib QD. Dose-escalation with intermittent dosing is ongoing. Out of the 18 patients evaluable for response, regression of target lesions was seen in six patients, with one confirmed partial response in a NSCLC patient. Median time to progression was 49 days (range 6–350). Reductions in pERK and pS6 levels were demonstrated in eight out of 13 paired tumor biopsies (mean H-score change -50% and -12%).

**Conclusions** Lapatinib and trametinib could be combined in patients with manageable toxicity. Although single agent full doses could not be reached in combination, preliminary signs of anti-tumor activity have been observed at tolerable dose-levels. The ongoing study explores intermittent dosing schedules to improve tolerability and exposure with a focus on NSCLC patients.

## INTRODUCTION

Approximately 20% of all human cancers carry mutations in the *KRAS* oncogene, including 90% of pancreatic cancers, 45% of colorectal cancers, and 35% of non-small cell lung cancers[1]. *KRAS* gain-of-function mutations cause continuous activation of the mitogen-activated protein kinase (MAPK) pathway, resulting in the development and progression of malignant cells. The high frequency of *KRAS* mutations in three of the most lethal tumor types makes targeting of these mutated proteins with small molecule tyrosine kinase inhibitors an attractive treatment strategy. However, despite extensive efforts over the past decades, effective KRAS inhibitors have not yet reached the clinic[2]. An alternative approach to target KRAS-driven tumors comprises inhibition of downstream effectors of KRAS, such as MEK or ERK. Although MEK inhibitors were found to be among the most active agents against *KRAS* mutant (*KRAS*m) cell lines, their effect was mostly cytostatic rather than cytotoxic, and the anti-tumor activity in xenograft models and patients has been limited.[3–5]

The underlying mechanism of intrinsic resistance to MEK inhibitors was elucidated by Sun *et al.* who demonstrated that upon MEK inhibition, MYC-dependent transcriptional upregulation of the human epidermal growth factor receptor (HER) 3 takes place. Subsequently, reactivation of downstream signaling pathways results in sustained activation of multiple tumorigenic mechanisms. As HER-3 requires formation of heterodimers with the epidermal growth factor receptor (EGFR) or HER-2 in order to activate downstream signaling, inhibition of EGFR and HER-2 in addition to MEK was sufficient to obtain synergistic anti-tumor activity *in vitro* and in xenograft models[6]. These findings provided a rationale for clinical evaluation of such a combination of targeted agents.

Therefore, a phase I dose-finding study was initiated, in which the dual EGFR/HER inhibitor lapatinib and the MEK inhibitor trametinib were combined in patients with *KRAS*m colorectal cancer (CRC), non-small cell lung cancer (NSCLC) or pancreatic cancer with the aim of defining the recommended phase II dose (RP2D) and the most tolerable regimen. Patients were also selected for absence of *PIK3CA* mutations to avoid treatment resistance via the PI3K-pathway. Lapatinib is an ATP-competitive dual tyrosine kinase inhibitor targeting EGFR and HER2, approved as standard of care for inhibiting HER2 activity in HER2-positive breast cancer[7–9]. Trametinib is a reversible, highly selective, allosteric MEK1 and MEK2 inhibitor, approved for the treatment of patients with metastatic *BRAF* mutant melanoma in combination with a BRAF inhibitor[10,11]. This manuscript provides an interim analysis of the ongoing clinical study.

## PATIENTS AND METHODS

### Patient population

In this investigator-initiated, single-center, open label phase I study, patients with histologically- or cytologically-confirmed advanced CRC, NSCLC or pancreatic cancer and a documented *KRAS* exon 2, 3 or 4 mutation and *PIK3CA* wildtype could be included. Eligible patients were 18 years or older, had an Eastern Cooperative Oncology performance status of 0 or 1, had a life expectancy ≥3 months, had measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, and had adequate bone marrow (absolute neutrophil count ≥1.5 x $10^9$/L, platelets ≥100 x $10^9$/L, hemoglobin ≥6.0 mmol/L) and organ function (total bilirubin ≤1.5 x upper limit of normal [ULN], aspartate aminotransferase and alanine aminotransferase ≤2.5 x ULN, serum creatinine ≤1.5 x ULN). Any treatment within four weeks prior to the first dose of study treatment was not allowed. Patients with symptomatic or untreated leptomeningeal disease were excluded, as well as patients with symptomatic brain metastasis, history of interstitial lung disease, pneumonitis or retinal vein occlusion, and patients previously treated with combinations of targeted agents known to interfere with EGFR, HER2, HER3, HER4 or MAPK- and PI3K-pathway components, including BRAF, MEK, ERK, PI3K, AKT and mTOR. The study protocol and all amendments were approved by regulatory authorities and the medical ethics committee of the Netherlands Cancer Institute. All patients gave written informed consent per Declaration of Helsinki recommendations. In December 2016, the study protocol was amended to restrict inclusion to NSCLC patients only, based on emerging preclinical and clinical evidence on preferential activity in this tumor type.

The study was registered at ClinicalTrials.gov (NCT02230553). GlaxoSmithKline Inc. and Novartis Pharma Inc. funded this study and provided study medication.

### Study design and procedures

The primary objective of this trial was to determine the recommended phase 2 dose (RP2D) and regimen of the combination of lapatinib and trametinib. Secondary objectives included characterizing safety and tolerability, assessing preliminary anti-tumor activity and pharmacodynamic effects, and evaluating the pharmacokinetic parameters of lapatinib and trametinib when given concomitantly. For this aim, patients were assigned to

dose-levels with varying lapatinib and trametinib doses starting at approximately 50% of their monotherapy doses, which is 750 mg lapatinib once daily (QD) and 1 mg trametinib QD, accounting for potentially synergistic toxicities. Dose-escalation followed an alternate escalation schedule with fixed increments according to a classical 3+3 design. Dose escalation decisions were based on the occurrence of dose-limiting toxicity (DLT) during the first 28 days (cycle one). Patients were considered evaluable for DLT if at least 75% of the assigned lapatinib and trametinib doses were administered in cycle one, or if a DLT had occurred. The RP2D was defined as the dose at which no more than one out of six patients experienced DLT during treatment cycle one. After assessing the RP2D of lapatinib plus trametinib at continuous dosing schedules, intermittent dosing schedules were initiated in order to optimize the exposure and tolerability. Study treatment was continued until disease progression, unacceptable treatment-related toxicity despite supportive measures and dose modifications, or investigator/patient decision to withdraw study consent.

Safety evaluations were performed at baseline and throughout the study and included physical examination, vital signs, routine laboratory assessments, electrocardiography, ophthalmic examination, and multigated acquisition (MUGA) scans to assess the left ventricular ejection fraction. Adverse events were recorded according to Common Terminology Criteria for Adverse Events version 4.0. DLTs were defined as adverse events or laboratory abnormalities occurring within the first 28 days of study treatment that meet at least one of the criteria described in supplementary table S1. Tumor response was assessed radiographically according to RECIST version 1.1. For the first 22 patients this was done every six weeks. After a protocol amendment, tumor response was assessed every eight weeks. Patients were evaluable for efficacy if at least one follow-up radiographic evaluation was performed.

**Pharmacokinetic and pharmacodynamic analysis**

For pharmacokinetic analyses, extensive blood sampling was performed in all patients. Serial blood samples were taken on the first day of cycle one and cycle two predose and 0.5, 1, 2, 4, 6, 8, and 24 hours after dosing. Plasma was isolated and stored at -80°C until analysis. Analysis was performed using a validated high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method. Briefly, lapatinib and trametinib were extracted from plasma by protein precipitation with a mixture of acetonitrile/methanol (1:1 v/v). Compounds were chromatographically separated using a Waters Xbridge BEH Phenyl column (50 x 2.1 mm ID, 5 µm particle size) and detection was performed using an API4000 tandem mass spectrometer equipped with a turbo ion spray interface, operating in the positive ion mode. Transitions from m/z 584 to 458 and m/z 622 to 497 were monitored for the detection of lapatinib and trametinib, respectively. Stable isotopically labelled internal standards were used for the quantification. The lower and upper limits of quantification were respectively 0.5 and 50 ng/mL for trametinib, and 50 and 5,000 ng/mL for lapatinib. Pharmacokinetic parameters were calculated in R using an in-house developed package for non-compartmental pharmacokinetic analyses (version 1.3)[12].

For pharmacodynamic analyses, tumor biopsies were taken before treatment, in the second week of treatment and if feasible upon treatment discontinuation. Phosphorylated (p) ERK and ribosomal pS6 (pS6-r) levels were measured by validated immunohistochemistry (IHC) staining methods and semi-quantitative H-scores (percentage of positive cells (0–100) multiplied by staining intensity (0–3)) were assessed by an independent pathologist who was blinded for sample identification. Tumor biopsy samples were fixed in formalin for 16–24 hours and embedded in paraffin subsequently. Immunohistochemistry of formalin-fixed paraffin-embedded tumor samples was performed on a BenchMark Ultra autostainer (Ventana Medical Systems). Briefly, paraffin sections were cut at 3 µm, heated at 75°C for 28 minutes and deparaffinised in the instrument with EZ prep solution (Ventana Medical Systems). Heat-induced antigen retrieval was carried out using Cell Conditioning 1 (CC1, Ventana Medical Systems) at 95°C for 32 and 64 minutes, for pS6-r and pERK1/2, respectively. pS6-r was detected using clone D68F8 (1:1000 dilution, 32 minutes at room temperature, Cell Signalling) and p-p44/42 MAPK (pERK1/2) (Thr202/Tyr204) using clone D13.14.4E (1:400 dilution, one hour at room temperature, Cell Signalling). pERK was detected using the UltraView Universal DAB Detection Kit (Ventana Medical Systems), while detection for pS6-r was performed using the OptiView DAB Detection Kit (Ventana Medical Systems). Slides were counterstained with hematoxylin.

**Statistical analysis**

Patient characteristics, efficacy and safety data were summarized using descriptive statistics. A paired t-test was used to determine the statistical significance of the pharmacodynamic modulation (i.e. pERK and pS6-r) in tumor tissue taken before study start and while on treatment. A two sample t-test assuming equal variances was used to determine statistical significance of the difference in exposure between lapatinib and trametinib doses. A linear regression analysis was performed to explore the correlation between exposure of lapatinib and trametinib in terms of AUC on cycle one day one and pERK modulation.

## RESULTS

### Patient disposition and characteristics

At the time of data cut off, May 1st 2017, 27 patients were enrolled in the study across five different dose-levels; 16 patients with colorectal cancer, eight patients with NSCLC and three patients with pancreatic cancer. The majority of these patients (n=25) had a mutation in *KRAS* exon 2 (codon 12 or 13), and two patients had an exon 4 (codon 146) *KRAS* mutation. The most frequently reported mutations were G12D (n = 8), G12V (n = 5) and G12A (n = 4). Also, G12C (n=3), G13D (n=2), A146V (n=2), G12R (n=2) and G12S (n=1) mutations were reported. Among the tumor types, G12D mutations occurred most frequently in NSCLC and CRC, while all three pancreatic cancer patients had different mutations (G12D, G12V and G12R). The majority of patients was pretreated with at least two prior lines of therapy for metastatic disease (table 1). At data cut off, all patients had discontinued study treatment, 19 patients due to progressive disease, four patients due to adverse events, and four patients due to patient refusal.

### Dose finding

Out of the 27 patients, 22 were evaluable for DLT. Five patients discontinued early (n=3) due to patient refusal or received less than 75% of the administrations planned in cycle 1 (n=2) due to adverse events not related to treatment. Dose-limiting toxicities were not observed in the first cohort of three patients on dose-level 1 (750 mg lapatinib QD, 1 mg trametinib QD), allowing escalation of trametinib. However, in the subsequent dose-levels 2 and 3, comprising 750 mg lapatinib QD plus 1.5 mg trametinib QD and 500 mg lapatinib QD plus 1.5 mg trametinib QD, respectively, dose-limiting toxicities were reported in two out of six and two out of three patients, respectively. Therefore, we enrolled an additional three patients on the initial dose-level. Finally, one DLT was observed amongst six patients which resulted in 750 mg lapatinib QD plus 1 mg trametinib QD being the RP2D. Dose-limiting adverse events were grade 3 diarrhea in dose-level 1, grade 3 rash and grade 3 aspartate aminotransferase elevation in dose-level 2, and grade 3 diarrhea and inability to receive at least 75% of the planned dose due to a grade 4 creatine phosphokinase (CPK) elevation in dose-level 3 (figure 1).

**Table 1**. Patient and disease characteristics at baseline.
*Abbreviations: ECOG PS, Eastern Cooperative Oncology performance status; KRAS, Kirsten rat sarcoma viral oncogene homolog.*

| | Patients (n = 27) | |
|---|---|---|
| Sex, n (%) | | |
| Female | 12 | (44%) |
| Male | 15 | (56%) |
| Age, mean (range), years | 60 | (43–75) |
| Tumor types, n (%) | | |
| Colorectal | 16 | (59%) |
| Non-small cell lung | 8 | (30%) |
| Pancreatic | 3 | (11%) |
| ECOG PS, n (%) | | |
| 0 | 14 | (52%) |
| 1 | 13 | (48%) |
| Number of prior treatment lines, n (%) | | |
| 1 | 4 | (15%) |
| 2 | 15 | (55%) |
| ≥ 3 | 8 | (30%) |
| KRAS mutation, n (%) | | |
| Exon 2 | 25 | (92%) |
| p.G12D | 8 | (30%) |
| p.G12V | 5 | (19%) |
| p.G12A | 4 | (15%) |
| p.G12C | 3 | (11%) |
| p.G13D | 2 | (7%) |
| p.G12R | 2 | (7%) |
| p.G12S | 1 | (4%) |
| Exon 3 | 0 | |
| Exon 4 | 2 | (7%) |
| p.A146V | 2 | (7%) |

Intermittent dosing regimens were subsequently initiated. A 5 days on/2 days off regimen was chosen to achieve a more gradual increase in plasma levels with short recovery periods. Dose-level 4 consisted of 750 mg QD in a 5 days on/2 days off regimen and trametinib 1.5 mg QD continuously. No DLTs were observed at this dose-level. Dose-level 5 with 750 mg lapatinib QD and 2 mg trametinib QD, both in a 5 days on/2 days off regimen, resulted in DLT in two out of four patients. DLTs consisted of nausea grade 3 resulting in a treatment delay >7 days in one patient and rash grade 3 resulting in treatment delay of >7 days and intake of <75% of the planned doses in another patient. Formally, the previous dose-level should have been expanded. However, because the investigators preferred a 5 days on/2 days off regimen for both agents in view of patient convenience, a new dose-level was opened consisting of 750 mg lapatinib and 1.5 mg trametinib both in a 5 days on/2 days off regimen. This dose-level was open for enrollment at the time of data cut-off.

**Safety**

As shown in table 2, the most frequent adverse events at least possibly related to treatment were skin toxicity (100%), diarrhea (81%), and fatigue (56%). These toxicities occurred mainly within the first weeks of treatment. Skin toxicity presented as acneiform, maculo-papular or papulo-pustular rash (89%), mainly on the face chest and back, hand-foot syndrome (15%), and as dry skin (10%). One grade 3 event of abscess-forming folliculitis was observed at dose-level 5. Preventive actions for skin toxicity were undertaken including the use of cetomacrogole cream and cream with sun protection factor. Diarrhea was predominantly grade 1-2 and was mostly manageable with standard supportive care. Diarrhea was the major cause of treatment interruption (n=4) or discontinuation (n=3). Treatment-related ocular toxicity was observed in four patients but did not cause interruption or discontinuation of study treatment. Five patients experienced a decrease in left ventricular ejection fraction (LVEF) of median 15% [range: 12-46%], including two patients with grade 3 events. One patient discontinued study treatment permanently due to a decreased LVEF from 70% at baseline to 24% after nine cycles that did not improve to more than 50% within four weeks of treatment interruption. The other patient with a 21% LVEF decrease had several treatment interruptions and one dose-reduction of lapatinib which allowed recovery to grade 2. All other LVEF events did not require treatment interruption.

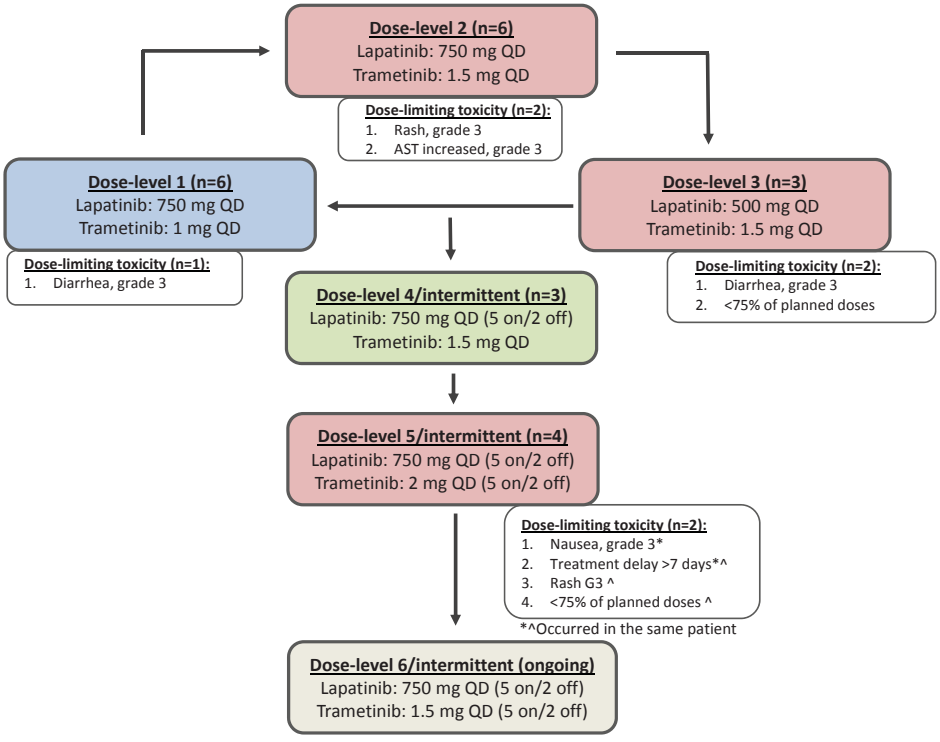


**Figure 1.** Dose-escalation cohorts and dose-limiting toxicities . *Abbreviations: QD, once daily; AST, aspartate aminotransferase.*

**Table 2.** Adverse events, at least possibly related occurring in ≥ 10% of patients, highest grade per patient.
*Abbreviations: CPK, creatine phosphokinase; ALT/AST, alanine aminotransferase / aspartate aminotransferase; LVEF, left ventricular ejection fraction; 5/2, 5 days on / 2 days off.*

| | Dose-level 1 (n =7) | | Dose-level 2 (n = 7) | | Dose-level 3 (n = 4) | | Dose-level 4 (n = 4) | | Dose-level 5 (n = 5) | | **Total (n = 27)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lapatinib QD*<br>*Trametinib QD* | *750 mg*<br>*1 mg* | | *750 mg*<br>*1.5 mg* | | *500 mg*<br>*1.5 mg* | | *750 mg (5/2)*<br>*1.5 mg* | | *750 mg (5/2)*<br>*2 mg (5/2)* | | | |
| Adverse Event, n % | *Grade 1/2* | *Grade 3* | *Grade 1/2* | *Grade 3* | *Grade 1/2* | *Grade 3* | *Grade 1/2* | *Grade 3* | *Grade 1/2* | *Grade 3* | **Grade 1-3** | |
| Any skin toxicity | 7 26% | | 5 19% | 3 11% | 4 15% | | 4 15% | | 4 19% | | **24** | **100%** |
| *Rash* | 5 19% | | 5 19% | 2 7% | 4 15% | | 4 15% | | 4 15% | | **24** | **89%** |
| *Hand-foot syndrome* | 2 7% | | 2 7% | | | | | | | | **4** | **15%** |
| *Dry skin* | 2 7% | | | | | | | | 1 4% | | **1** | **4%** |
| *Follicultitis* | | | | 1 4% | | | | | | | **1** | **4%** |
| Diarrhea | 4 14% | 2 7% | 5 19% | 1 4% | 1 4% | 1 4% | 3 11% | 3 11% | 2 7% | | **22** | **81%** |
| Fatigue | 6 22% | | 4 15% | 1 4% | 2 7% | | 1 4% | | 1 4% | | **15** | **56%** |
| Nausea | 3 11% | | 2 7% | | 2 7% | | 2 7% | | 4 15% | | **13** | **48%** |
| Vomiting | 1 4% | | 4 15% | | 1 | | 2 7% | | 4 15% | | **12** | **44%** |
| ALT/AST increased | 1 4% | | 4 15% | | 2 7% | | | | 1 4% | | **8** | **30%** |
| CPK increased | 2 7% | | 2 7% | | | | 1 4% | | | | **5** | **19%** |
| Mucositis | | | 4 15% | | 1 4% | | | | | | **5** | **19%** |
| LVEF decreased | 1 4% | 2 7% | 2 7% | | | | | | | | **5** | **19%** |
| Ocular toxicity | 3 11% | 1 4% | 1 4% | | | | | | | | **4** | **15%** |
| *Dry eyes* | 1 4% | | 1 4% | | | | | | | | **2** | **7%** |
| *Edema eyes* | 2 7% | 1 4% | | | | | | | | | **2** | **7%** |
| *Uveitis* | 1 4% | | | | | | | | | | **1** | **4%** |
| Anorexia/dysgeusia | 1 4% | | 1 4% | | | | | | 1 4% | 1 4% | **4** | **15%** |
| Edema | 1 4% | | 1 4% | | 1 4% | | | | 1 4% | | **4** | **15%** |
| Weight loss | 2 7% | | 1 4% | | | | | | 1 4% | | **3** | **11%** |
| Hypertension | 1 4% | | 1 4% | | | | 1 4% | | | | **3** | **11%** |

**Pharmacokinetic analysis**

For lapatinib and trametinib, day one and steady state $AUC_{0-24}$, $T_{max}$, and $C_{max}$ at each dose-level are summarized in table 3. Standard pharmacokinetic parameters are in line with previously reported data[13,14]. Trametinib and lapatinib were absorbed with a time to maximum plasma concentration ($T_{max}$) of approximately 4 hours for lapatinib (750 mg and 500 mg), and 1 to 2 hours for trametinib (1 mg and 1.5 mg). Individual day one and steady-state (day 29 or day 26 in the 5/2 dose-levels) plasma concentration profiles at each dose are given in figure 2. Repeated lapatinib dosing resulted in an approximate 1.7-fold increase in exposure at steady-state relative to day one, with a mean area under the plasma concentration time curve from time zero to 24 hours ($AUC_{0-24}$) of $15{\cdot}10^3$ ng*h/mL (between subject coefficient of variation [CV%], 52%) at day one and $26{\cdot}10^3$ ng*h/mL (CV 63%) at steady-state. Compared to 750 mg continuously, intermittent dosing of lapatinib and de-escalation to 500 mg resulted in lower exposure in cycle 2, although not statistically significant. The unique exposure profile of trametinib, including a small peak-to-trough-ratio, long effective half-life and low interpatient variability was recognized in our data as well. Trametinib exposure at day one was significantly increased ($AUC_{1.5mg}$ vs. $AUC_{1mg}$; $p$=0.02 and $AUC_{2mg}$ vs. $AUC_{1\ mg}$; $p$=0.02*)* by escalations of trametinib from 1 mg, to 1.5 mg and 2 mg. However, at steady state, exposure was not significantly different between the dose-levels with regards to AUC and plasma levels (figure 2). Trametinib accumulated by eight-fold after multiple administrations.

**Table 3.** Pharmacokinetic parameters of lapatinib and trametinib at day 1 and steady-state. **Pharmacokinetic samples were taken at the last day of cycle 1 on which lapatinib and trametinib were given concurrently, i.e. cycle 1 day 26. Data are listed as geometric mean. $AUC_{0-24}$ and $C_{max}$ data are given as mean (CV%). 5/2, 5 days on 2 days off.*

| **Dose-level** | **1** | **2** | **3** | **4*** | **5*** | **Lapatinib** | **Trametinib** |
|---|---|---|---|---|---|---|---|
| *Lapatinib QD* | 750 mg | 750 mg | 500 mg | 750 mg (5/2) | 750 mg (5/2) | **All 750 mg** | **All 1.5 mg** |
| *Trametinib QD* | 1 mg | 1.5 mg | 1.5 mg | 1.5 mg | 2 mg (5/2) | **doses** | **doses** |
| **Lapatinib** | *Cycle 1 Day 1* | | | | | | |
| *Mean* | n = 7 | n = 6 | n = 3 | n = 4 | n =5 | **n = 17** | - |
| $C_{max}$ ($\cdot10^3$ ng/mL) | 1.1 (46) | 1.4 (71) | 0.99 (36) | 0.67 (44) | 1.6 (18) | **1.2 (54)** | |
| $T_{max}$ (h) | 3.5 | 3.6 | 4.7 | 3.1 | 2.8 | **3.3** | |
| $AUC_{0-24h}$ ($\cdot10^3$ ng*h/mL) (%CV) | 13 (48) | 17 (63) | 13(25) | 10 (28) | 20 (25) | **15 (52)** | |
| **Lapatinib** | *Cycle 2 Day 1* | | | | | | |
| *Mean* | n = 3 | n = 3 | n = 1 | n = 2 | n = 2 | **n = 9** | - |
| $C_{max}$ (ng/mL) | 2.1 (60) | 1.7 (64) | 0.80 | 0.81 (8) | 2.1 (36) | **1.8 (61)** | |
| $T_{max}$ (h) | 3.9 | 3.3 | 4.0 | 3.0 | 4.0 | **3.6** | |
| $AUC_{0-24h}$ ($\cdot10^3$ ng*h/mL) (%CV) | 33 (56) | 23 (64) | 12 | 11 (18) | 30 (19) | **26 (63)** | |
| **Trametinib** | *Cycle 1 Day 1* | | | | | | |
| *Mean* | n = 6 | n = 6 | n = 3 | n = 4 | n = 5 | - | **n = 13** |
| $C_{max}$ (ng/mL) | 2.7 (52) | 4.0 (36) | 3.9 (32) | 2.9 (27) | 5.7 (59) | | **3.6 (34)** |
| $T_{max}$ (h) | 0.9 | 2.3 | 2.0 | 1.9 | 1.0 | | **2.0** |
| $AUC_{0-24h}$ ($\cdot10^1$ ng*h/mL) (%CV) | 2.1 (43) | 3.4 (39) | 3.7 (19) | 2.7 (24) | 3.9 (32) | | **3.2 (26)** |
| **Trametinib** | *Cycle 2 Day 1* | | | | | | |
| *Mean* | n = 4 | n = 3 | n = 1 | n = 2 | n = 2 | - | **n = 6** |
| $C_{max}$ (ng/mL) | 13 (27) | 15.9 (50) | 9.1 | 17.3 (41) | 16.5 (2) | | **15.2 (39)** |
| $T_{max}$ (h) | 1.14 | 2.5 | 8 | 2.0 | 3.0 | | **3.2** |
| $AUC_{0-24h}$ ($\cdot10^1$ ng*h/mL) (%CV) | 23 (26) | 27 (46) | 19 | 28 (9) | 36 (0.3) | | **26 (34)** |

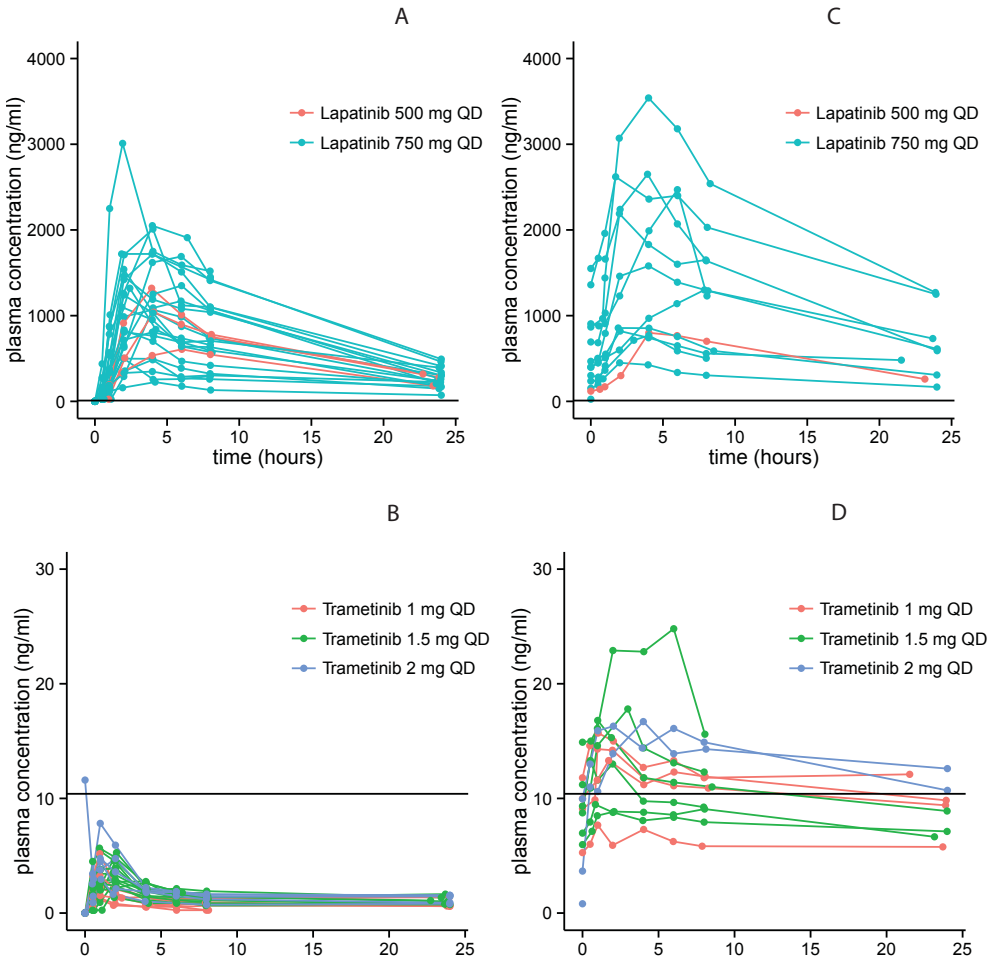


**Figure 2.** Pharmacokinetic profiles of lapatinib and trametinib.
*Mean plasma concentration curves for lapatinib and trametinib, per dose at day 1 (A, B) and at steady-state, at cycle 2 day 1 or the last day of concomitant use of both drugs in cycle 1 after at least 3 weeks of administration (C, D). The solid line indicates target levels of 9.43 ng/ml for lapatinib[24] and 10.4 ng/ml for trametinib[23].*

**Antitumor activity**

Eighteen patients were evaluable for efficacy; nine patients did not reach the first radiographic evaluation due to withdrawal of consent (n=4), adverse events (n=3), clinical progressive disease (n=1) or a lack of evaluable target lesions (n=1). Out of the evaluable patients, one patient (6%) achieved a confirmed partial response, nine (50%) had stable disease as best response, and eight (44%) had progressive disease at the first response evaluation. Although only one partial response (> 30% decrease of tumor volume) was observed, tumor regression was achieved in six patients at dose-levels 1, 2 and 5. Three out of eight patients with NSCLC had tumor regression, including one confirmed partial response (figure 3). The median overall time on treatment was 49 days (range 38–350). Separated by tumor type, time on treatment was 168 days (range 59–350) for patients with NSCLC, 59 days (range 38-133) for CRC patients, and 85 days for the evaluable patient with pancreatic cancer (figure 4).

**Pharmacodynamic analysis**

Paired tumor biopsies were obtained at baseline and on treatment from 17 patients. In total, 13 paired tumor biopsies contained sufficient numbers of tumor cells (>10%) and were considered evaluable for pharmacodynamic analysis. The mean pERK H-score modulation was -50% ($p$=0.04), with eight out of 13 patients showing reduction in pERK intensity-score. In eight patients pS6 intensity-score was decreased upon

treatment and the mean pS6 modulation was -12% ($p$=0.2). Figure 5 illustrates how pERK and pS6 modulation was visualized. In seven patients, reduction of both pERK and pS6 were observed (figure 6 A and B).

In 12 patients, the biopsied lesion could also be evaluated radiographically which allowed exploring the correlation between pERK modulation and response of the corresponding lesion after six weeks of treatment. In four patients, modulation of pERK coincided with reduction of the lesion volume. In three patients, the lesion had progressed after six weeks of treatment despite pERK modulation in the second week of treatment (figure 6C) and in two patients an increase of lesion volume matched with an increase of pERK H-scores. There was no clear correlation between exposure to lapatinib or trametinib and modulation of pERK and pS6 (data not shown).

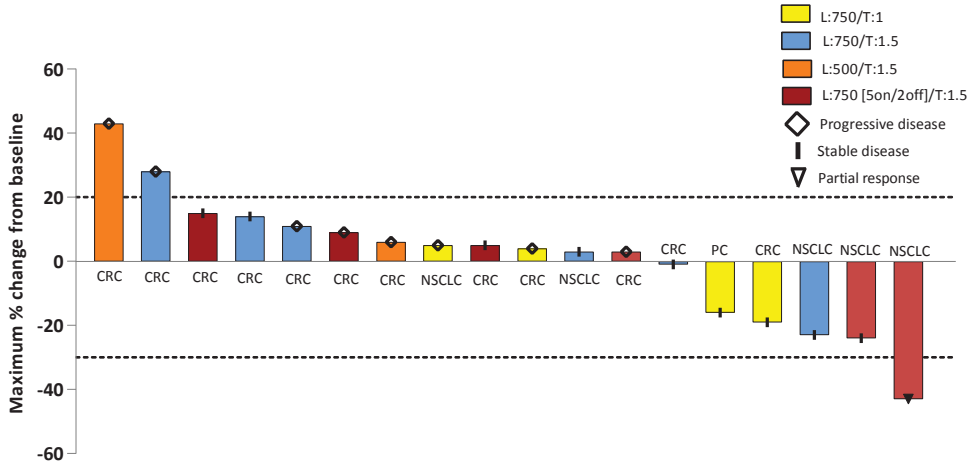


**Figure 3.** Maximum percentage change in sum of target lesion size from baseline by dose-level, including response evaluation by RECIST. *Abbreviations: L, lapatinib; T, trametinib; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; PC, pancreatic cancer.*

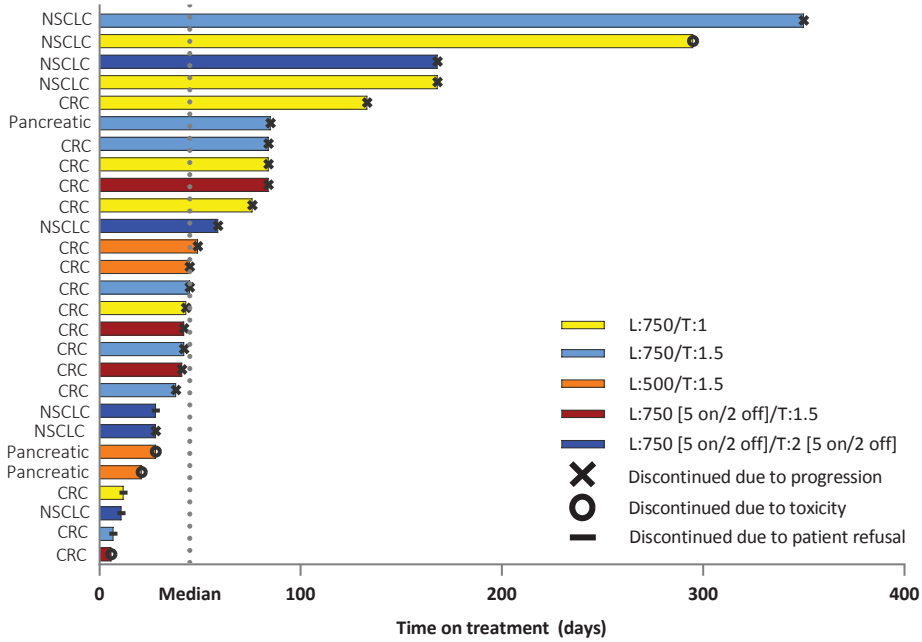


**Figure 4.** Time on treatment, by dose-level. Bars represent duration on treatment by dose-level, with the reason for end of treatment displayed at the end of each bar. *Abbreviations: L, lapatinib; T, trametinib; QD, once daily; CRC, colorectal cancer; NSCLC, non-small cell lung cancer; Pancreatic, pancreatic cancer.*

## DISCUSSION

Our preliminary findings show that trametinib and lapatinib can be combined with manageable toxicity, at approximately 50% of their single agent doses. In a continuous dosing regimen, 750 mg lapatinib plus 1 mg trametinib was declared the RP2D. The RP2D in an intermittent administration regimen is still to be determined. So far, 750 mg in a 5 days on/2 days off regimen can be combined with 1.5 mg QD of trametinib continuously with acceptable toxicity. On this intermittent dose-level, three grade 3 toxicities were observed compared to eight among patients who received 750 mg lapatinib continuously combined with 1.5 mg trametinib. In previous studies, lapatinib was well tolerated at doses up to 1,600 mg QD, and the RP2D of trametinib was established at 2 mg QD[13–15]. In combination, full single-agent doses could not be administered due to the occurrence of dose-limiting events including rash, diarrhea and liver enzyme elevation. However, previous studies show that even at 50% of the monotherapy doses, target engagement and clinical responses can be achieved in patients with *BRAF*m melanoma and EGFR-expressing and/or HER2-overexpressing breast cancer, respectively[13–15]. This was confirmed by the target engagement observed in tumor biopsies. Relevant suppression of the MAPK-pathway was achieved, which was indicated by a reduction of pERK in the majority of patients and in all histological tumor types. In addition, the PI3K-signaling pathway was suppressed based on pS6-r modulation.

Although the pharmacodynamic effects were promising, a correlation with clinical activity (e.g. response rate, time on treatment) could not be confirmed. One possible explanation for this, is that pERK suppression may be transient or insufficient and tumor cells find escape mechanisms to reactivate ERK phosphorylation or another preferred survival pathway quickly after the on-treatment biopsy. This is confirmed by the finding that pERK modulation, measured in the second week of treatment, did not clearly correlate with radiologic regression of the corresponding lesion, evaluated after six weeks of treatment, in the majority of patients (figure 6C). Secondly, inter-metastasis heterogeneity and sensitivity within patients may play a role. Indeed, the biopsied lesions in two patients showed radiological changes of -11% and -4%, whereas the sum of target lesion diameters had increased. In these lesions, pERK and pS6-r scores decreased upon treatment with -56% and -88% and -100% and -50% respectively. Another explanation for limited clinical efficacy lies in the pharmacokinetics. In general, pharmacokinetic data obtained in this study was in line with single agent data from previous studies[13,14], indicating the absence of an obvious pharmacokinetic drug-drug interaction between lapatinib and trametinib. The unique pharmacokinetic profile of trametinib, with a long effective half-life and small peak-to-trough ratio allows constant target inhibition with relatively low $C_{max}$[10]. However, at 1 and 1.5 mg, the preclinical plasma concentration target of 10.4 ng/mL (i.e. the estimated mean inhibitory concentration at which 50% growth occurs in *BRAF*m melanoma cell lines)[15], was reached for only 50% of the patients and only 15 hours of the 24-hour dosing interval at steady state. In a previous phase I study with single agent trametinib, Infante *et al.*[13] showed that the preclinical target concentration was exceeded during the entire dosing interval only at doses of 2 mg and higher.

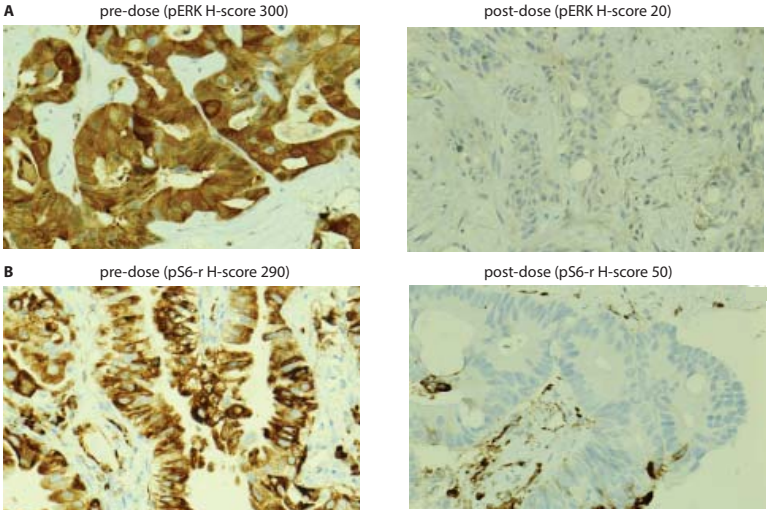


**Figure 5.** Representative immunohistochemistry sections of pERK (A) and pS6 (B) stainings in tumor biopsies (zoom 40x).

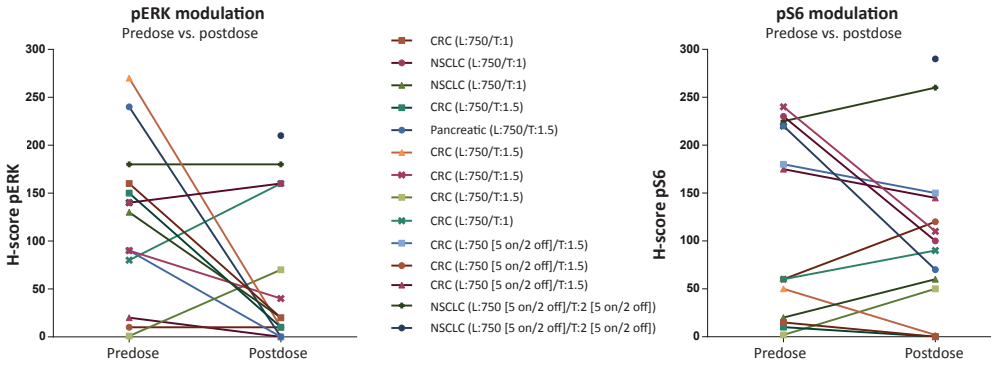


**Figure 6A and 6B.** Pharmacodynamic modulation in paired tumor biopsies.
*Tumor biopsies were obtained pre-dose (up to 1 week prior to treatment initiation) and post-dose (15–18 days after treatment start). Biopsy samples were analyzed for pERKThr202/Tyr204 and pS6-r by immunohistochemistry. Pre- and post-dose H-scores of pERK (A) and pS6 (B) are shown per individual.*

As robust MEK and MAPK pathway inhibition is crucial for optimal anti-tumor activity and because higher trametinib doses yielded stronger pERK suppression and improved clinical outcome in previous studies with trametinib as single agent or in combination[16] in patients with *BRAF* or *NRAS* mutated melanoma[13,15], dose-escalation was considered desirable. To allow increased exposure to lapatinib and trametinib with preserved tolerability, intermittent dosing regimens were initiated starting with continuous trametinib in combination with intermittent lapatinib, followed by another dose-level with intermittent administration of both drugs. This strategy was supported by *in vitro* data from our institute, demonstrating that sequential administration of concurrent MEK and EGFR-HER2 inhibition resulted in similar fractions of apoptotic cells in *KRAS*m cell lines as with concurrent administration (Bernards, unpublished data). Dose-escalation in these intermittent regimens was ongoing at the time of data cut off.

As expected, given the overlapping toxicity profiles of lapatinib and trametinib, skin-related toxicity and diarrhea were the most common treatment-related adverse events. In the majority of patients, early recognition and adequate supportive care was sufficient to make these effects manageable. Reductions in LVEF are common with MEK inhibitors and have been reported with lapatinib as well. During our study, five occurrences of LVEF reduction were reported. One patient experienced a LVEF reduction from 70% at baseline to 45% after five cycles of study treatment. As the LVEF recovered to >50% within two weeks upon treatment interruption, the patient continued on the same dose-level. However, after nine cycles LVEF had decreased to 24%. Because treatment interruption did not result in improvement of LVEF to ≥ grade 1 within four weeks, the patient discontinued study treatment permanently. Three months after study discontinuation, LVEF had recovered to >50%.

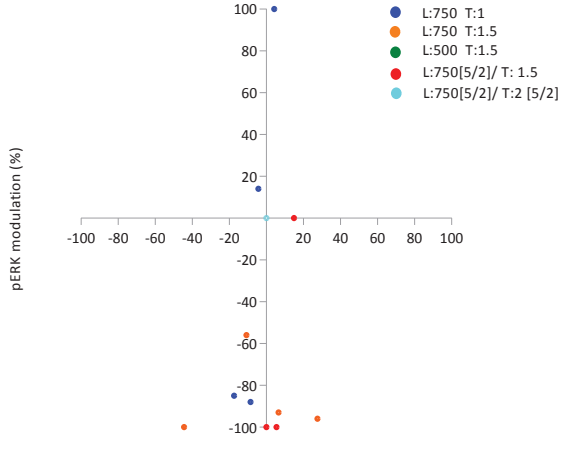


**Figure 6C.** The correlation between pERK modulation and volume change of the biopsied lesion are plotted for individual patients per dose-level.
*Abbreviations: CRC, colorectal cancer; NSCLC, non-small cell lung cancer; L, lapatinib; T, trametinib; 5/2, 5 days on, 2 days off regimen.*

Unfortunately, at tolerable doses, the anti-tumor activity obtained with trametinib plus lapatinib in patients with *KRAS*m malignancies was limited, with only one confirmed partial response so far. Previously, van Geel *et al.* demonstrated promising clinical activity with a combination strategy for patients with *BRAF*m CRC[17]. This combination therapy was based on a preclinical synthetic lethality drug screen showing synergistic activity between BRAF inhibition and an anti-EGFR directed antibody[18]. The exact same screening method identified dual EGFR-HER2 inhibitors to synergize with MEK inhibitors in *KRAS*m cells[6]. However, whereas in the BRAF setting clinical responses were achieved already at low BRAF inhibitor doses, this was not the case in this study in *KRAS* mutant tumors. Remarkably, three out of five patients with NSCLC achieved regression of target lesions, including one confirmed partial response. Based on this finding, we hypothesized that there may be a difference in sensitivity between NSCLC and CRC and pancreatic cancer. This hypothesis was supported by the time-on-treatment data, showing a median disease stabilization time of six months in the NSCLC patients compared to two months in patients with CRC and three months in the single pancreatic cancer patient. Previous studies suggested a difference in sensitivity to MEK inhibition between NSCLC and CRC as well. Hochster *et al.* demonstrated marginal additional benefit for adding a MEK inhibitor to second-line irinotecan in patients with *KRAS*m CRC[19], whereas Jänne *et al.* showed that the overall response rate in patients with *KRAS*m NSCLC could be improved by adding the MEK inhibitor selumetinib to second-line treatment with docetaxel, although no significant effect on the progression-free survival and overall survival was observed[5,20]. Additional work planned in our study therefore includes trying to elucidate the underlying biological mechanism of this difference in sensitivity and to confirm the preliminary signals of preferential activity in NSCLC. Evaluation of additional potential biomarkers, including heregulin, HER2 and HER3 protein expression levels, may be relevant for that matter. Also, RNA and DNA sequencing will be performed on baseline and on-treatment tumor material to gain insight in changes in protein expression and mutation profiles upon treatment. Furthermore, given the primarily cytostatic effect of MEK inhibitors in *KRAS*m tumors[21], it may be interesting to investigate markers for apoptosis (e.g. Bcl-xL, caspase 3), and to explore the potential of adding anti-apoptotic protein inhibitors such as navitoclax[22].

Taken together, our study established the recommended phase 2 dose for the combination of lapatinib and trametinib when given concurrently on a continuous dosing schedule. We provided evidence of pharmacodynamic effects in *KRAS*m tumor tissue and we demonstrated preliminary clinical anti-tumor activity in patients with *KRAS*m NSCLC. Data from additional patients is needed to confirm these signs of efficacy, and intermittent dosing is currently being explored in order to optimize tolerability and anti-tumor activity in this ongoing study.

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## SUPPLEMENT

**Table S1.** Criteria for defining dose-limiting toxicities. *Abbreviations: AST, aspartate aminotransferase; ALT alanine aminotransferase; ULN, upper limit of normal; CTCAE, Common Terminology Criteria for Adverse Events; LLN, lower limit of normal; DLT, dose limiting toxicity*

| Toxicity | DLT definition |
|---|---|
| Hematologic | • Grade 4 neutropenia for ≥5 days<br>• Grade ≥3 febrile neutropenia<br>• Grade 4 anemia<br>• Grade 4 thrombocytopenia |
| Non-hematologic | • AST >5X ULN OR ALT >3X ULN AND bilirubin >2X ULN (after exclusion of disease progression and/or bile duct obstruction)<br>• Grade ≥3 nausea, vomiting or diarrhea in the presence of maximal supportive care<br>• Grade ≥2 peripheral sensory or motor neuropathy<br>• Grade ≥3 clinically significant toxicity related to study treatment, other than those listed above, with the following exceptions:<br>  • Electrolyte disturbances that respond to correction within 24 hours<br>  • Grade 3 hypertension that is adequately controlled by the addition of up to 2 additional antihypertensive medications<br>  • Grade 3 pyrexia that does not result in study discontinuation |
| Cardiac | • Ejection fraction < LLN with an absolute decrease of >10% from baseline with confirmation within 14 days |
| Other | • Inability to receive ≥75% of scheduled doses in treatment period due to toxicity related to study treatment<br>• Treatment delay of >7 days due to study treatment-related toxicity<br>• Grade 2 or higher toxicity that occurs beyond 28 days which in the judgment of the investigator is a DLT |

## Chapter 2.4

**Quantification of the exposure-toxicity relationship of combined MEK and pan-HER inhibitors in patients with *KRAS* mutated tumors**

*Manuscript in preparation*

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Alwin D.R. Huitema

**ABSTRACT**

**Aim** For patients with Kirsten rat sarcoma viral oncogene homolog (KRAS) mutated (m) tumors, the combination of MEK and pan-HER inhibitors provides a promising treatment option based on preclinical research. Three ongoing dose-finding studies are investigating this concept with the combinations of afatinib-selumetinib, dacomitinib-PD0325901 and lapatinib-trametinib. Preliminary results show that toxicity limits dose-escalation to doses close to single agent doses. Insight in the relationship between exposure and toxicity could help in determining the optimal doses. Therefore, we aimed to establish a pharmacokinetic (PK)- toxicodynamic (TOX) model to quantify the relationship between exposure to MEK and pan-HER inhibitors and the occurrence of dose-limiting toxicities (DLTs).

**Methods** PK was linked to toxicity using an effect compartment model. Inputs in the effect compartment were plasma concentrations of each MEK and pan-HER inhibitor normalized to literature-based trough levels at steady-state in monotherapy use, to account for inter-drug differences in the plasma concentrations. DLT (0 or 1) was modelled using a logistic regression model based on the amount of drug in the effect compartment.

**Results** The final PK-TOX model described the relationship between the cumulative area-under-the-plasma concentration-time-curve (AUC) and the probability of DLT. The probability of developing a DLT during the first 28 days of treatment was related to cumulative exposure by a logit-transformed logistic regression model. The separate contribution of drug class or individual drug on overall toxicity of the different compounds could not be identified. The maximum estimated probability of DLTs increased from 10% in week 1 to 59% in week 4, with an observed incidence of DLTs of 24%.

**Conclusion** We provide a framework to combine data from three different trials into a single PK-TOX model. Our PK-TOX model could not discriminate between the impact of exposure to MEK and pan-HER inhibitors on the probability of developing DLTs. Hence, decisions on dose modifications in the clinic can only be made on an individual basis, depending on the individual dose, drug and combination.

## INTRODUCTION

Persistent activation of the Ras-Raf-MEK-ERK mitogen-activated-protein-kinase (MAPK) signaling pathway is frequently observed in human cancers and is associated with sustained malignant cell growth and proliferation. Often, mutations in the Kirsten rat sarcoma viral oncogene homolog (KRAS) protein underlie this persistent pathway activation. Mutations in the *KRAS* gene occur as frequently as 45% in colorectal cancer (CRC), 35% in non-small cell lung cancer (NSCLC) and 90% in pancreatic cancer (PC)[1]. Targeting the KRAS protein or other proteins in the MAPK pathway provides an attractive treatment option for these groups of patients. However, attempts to inhibit KRAS-induced cell growth with Raf, MEK or ERK inhibitors have not resulted in sustained clinical responses so far[2–5]. Hence, a high unmet medical need exists for patients with *KRAS* mutant (*KRAS*m) tumors.

Preclinical experiments have shown that combined inhibition of MEK and multiple (pan-) human epidermal growth factor receptors (HER1, HER2, HER3, HER4) can lead to complete suppression of cell growth of *KRAS*m cell lines[5]. The underlying mechanism is that upon MEK inhibition, overexpression of HER, in particular HER2 and HER3, occurs which re-activates not only the MAPK-pathway but also the phosphoinositide 3-kinase (PI3K)-AKT pathway[6] (figure 1). This resistance mechanism can be overcome by combining MEK and pan-HER inhibitors[7]. Based on this preclinical evidence, three clinical trials were initiated in which different combinations of MEK and pan-HER inhibitors were administered. In these on-going trials, patients with *KRAS*m colorectal cancer, non-small cell lung cancer and pancreatic cancer were treated with escalating doses of the dual combinations of afatinib-selumetinib, lapatinib-trametinib or dacomitinib-PD-0325901 in order to determine the recommended phase II dose and regimen of the combinations[8–10].

Preliminary unpublished results indicate that toxicities limit dose-escalation and clinical use of these agents, which was previously also reported for the combination of MEK and HER1 (EGFR) inhibitors[11]. Frequently observed dose-limiting toxicities (DLTs) included diarrhea, nausea, skin toxicity, dehydration and liver damage, which is in line with the known safety profiles of single agent use[12–17]. Particularly skin toxicity and diarrhea are well known class-effects of both MEK and HER inhibitors. In previous (single-agent) phase I studies, skin toxicity occurred in about 70% of the patients on MEK as well as HER inhibitors, and diarrhea occurred in 80% of patients on MEK inhibitors versus 50% on HER inhibitors. This explains the high incidence of these toxicities when combining MEK and HER inhibitors. In an interim analysis of the three ongoing clinical studies with the combinations, 93% of patients experienced skin toxicity and 84% diarrhea, which was dose-limiting in 4% and 6%, respectively. Also nausea and fatigue are known overlapping toxicities (40%-50% on MEK or HER inhibitors as single agents[12,16,18–21]), which is reflected in our data as well: 40% of patients experienced nausea and fatigue. Upon the occurrence of a DLT, dose-reduction of either or both drugs is required to diminish toxicity. However, due to the overlap in the type of toxicities related to MEK and pan-HER inhibitors, it is difficult to decide which of the drugs in the combination should be administered at a reduced dose. To investigate whether it is more rational to either de-escalate MEK or pan-HER inhibitor doses in these situations, the here reported pharmacokinetic (PK)- toxicodynamic (TOX) model was developed. We aimed to relate the exposure to the MEK inhibitors selumetinib, trametinib and PD-032901 and pan-HER inhibitors afatinib, lapatinib and dacomitinib to the probability of encountering DLTs. Ultimately, this could provide supportive evidence for dose- and schedule-selection in patients treated with these and comparable drug combinations.

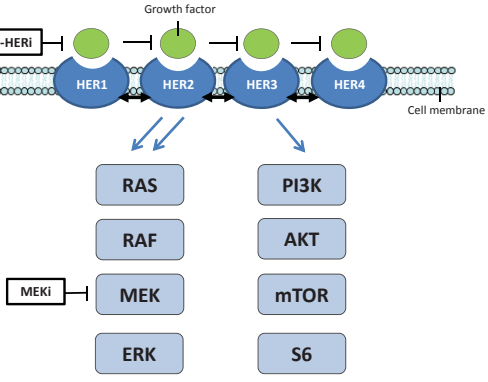


**Figure 1.** Schematic overview of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathway.

## METHODS

### Clinical studies

In three separate clinical studies, patients with *KRAS*m colorectal cancer, non-small cell lung cancer and pancreatic cancer were treated with one of the MEK inhibitors selumetinib, trametinib or PD-0325901 in a dual combination with one of the pan-HER inhibitors afatinib, lapatinib or dacomitinib, respectively. Doses were escalated according to pre-specified criteria, starting with 20-50% of the recommended monotherapy doses for each agent up to 75%-100% of the monotherapy doses. Dose-escalation was limited by the occurrence of dose-limiting toxicities (DLTs) during the first 28 days of treatment. Pre-defined criteria for DLTs were given in the study protocols and were consistent throughout the three studies, graded according to Common Terminology Criteria for Adverse Events version 4.0. DLTs were defined as severe neutropenia, anemia or thrombopenia (grade 3 for >5 days or grade 4), grade ≥ 3 nausea, vomiting or diarrhea in the presence of maximal supportive care, grade ≥2 neuropathy, severe liver enzyme elevations (aspartate aminotransferase (AST), alanine aminotransferase (ALT)), >10% decrease in left-ventricular ejection fraction, significant treatment delay (>7 days of delay or administration of <75% of planned doses in cycle 1), any other non-hematological toxicity grade ≥3, or any grade 2 toxicity which was considered a DLT in the judgement of the investigator. The clinical studies were conducted at the Netherlands Cancer Institute and three other hospitals in the Netherlands according to good clinical practice (GCP) guidelines and were registered at ClinicalTrials.gov as NCT02039336, NCT02450656, NCT02230553. All patients signed informed consent prior to start according to the Declaration of Helsinki. The PK-TOX model was fitted based on data from 84 patients among whom 20 encountered a DLT. In total, 25 DLTs were observed, indicating that some patients experienced more than one DLT.

### PK data

The occurrence of DLTs was evaluated at minimum at day 1, 2, 8, 15, 21 and 28 and in case of DLT at additional time points. Plasma concentration levels were measured in venous blood obtained by extensive pharmacokinetic sampling on day 1 (predose and 7 or 8 times postdose) and single pre-dose sampling on day 2, 4, 7, 15, 21 and 28. A combined validated assay was used to determine total drug concentrations in the Good Laboratory Practice Certified Bioanalytical Laboratory of the Netherlands Cancer Institute. Lower and upper limits of quantifications (LLoQs -ULoQs) were 0.5 – 50 ng/mL for dacomitinib, trametinib and afatinib, 5 – 500 ng/mL for PD-0325901 and selumetinib and 50 – 5,000 ng/mL for lapatinib. Concentrations below the LLoQs were included in the dataset as LLoQ/2 for any post-dose timepoint or as 0 if the time since the last administration exceeded five times the half-life of the compound.

### Model development and evaluation

For each compound, a PK model was developed based on the measured drug concentrations, taking into account all dose-interruptions based on real intake data from patient diaries and electronic patient dossiers. The PK models were accepted if successful minimization was reached, parameters and relative standard errors (RSE) could be estimated and weighted residuals were within acceptable ranges and without trends. Visual model evaluation was performed by visual predictive checks and by several other goodness-of-fit plots. From these PK models, individual plasma drug concentrations during the DLT observation period of 28 days were generated and used in the PK-TOX model using individual parameter estimations. For each drug, plasma concentrations were normalized to literature-based trough levels at steady-state ($C_{trough,ss}$) of monotherapy use[12,13,15,16,20,22] to account for inter-drug differences in the expected plasma concentrations and to allow merging of the data from the three trials. It was decided to normalize to $C_{trough}$ levels rather than to half maximal inhibitory concentrations ($IC_{50}$) values because $C_{trough}$ levels were more consistent in literature and were considered to reflect the differences in effective plasma concentrations better than $IC_{50}$ values only. The normalized concentration-time data of each compound was used as input in an effect compartment, which was subsequently linked to the probability of DLT. This was based on the hypothesis that each compound contributed equally to the probability of toxicity. Subsequently, a scaling factor was included on the MEK inhibitor to account for differences between classes (HER inhibitor versus MEK inhibitor) in their contribution to overall probability of DLT. First-order elimination from the effect compartment was initially included in the model. The PK-TOX models were evaluable only if successful minimization was reached with plausible and precise parameter estimates. Models were considered significantly improved in case of decreases in objective function values (OFVs) meeting the significance level of $p<0.01$ (with 1 degree of freedom this corresponds to a decrease in OFV >6.6) with lower or equal relative standard errors to the parameter estimates[23–25]. The PK-TOX model was considered successful if all of the above requirements were met and if it could be applied on the data of the three clinical studies as well as the combined data. For model-evaluation, the cumulative exposure and the maximum probability per week of treatment was extracted from the model output. This was plotted together with the observed number of patients with and without DLT in every week of treatment.

**Software**

Model estimations were performed using NONMEM (version 7.3.0, ICON Development Solutions, Ellicott City, MD, USA[23]) together with Pirana (version 2.9.4) as graphical interface[26]. R version 3.03 was used for data processing and graphical presentations[27]. For the PK modelling, the first order conditional estimation option with interaction (FOCE-I) was used. Individual Bayesian parameter estimates were generated using the POSTHOC option of NONMEM. For the modelling of probability of DLT, FOCE was used in combination with the LAPLACE and LIKE options.

## RESULTS

**PK-TOX model**

Parameter estimates of the PK models for each of the six drugs can be found in Appendix 1. The final model relating the drug exposure to the probability of DLTs is schematically shown in figure 2.

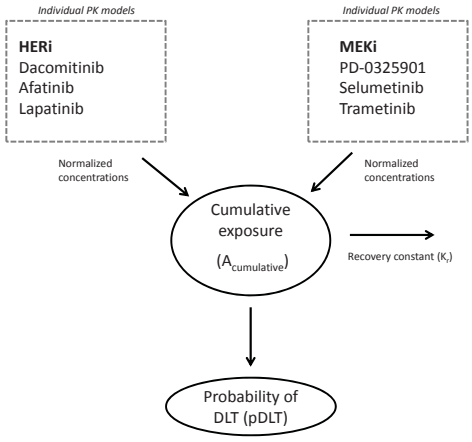


**Figure 2.** Schematic presentation of the pharmacokinetic (PK) – toxicodynamic (TOX) model for MEK and HER inhibitors (i). *DLT, dose-limiting toxicity.*

The probability of a DLT was modelled using the following equations:

$$\frac{dA_{cumulative}}{\mathrm{dt}} = C_{HER} + S * C_{MEK}$$

$$f = B_0 + B_1 * (A_{cumulative}/1000)$$

$$P_{DLT} = \frac{\exp(f)}{1 + \exp(f)}$$

Where $dA_{cumulative}$ represents the effect compartment, being cumulative exposure to MEK and pan-HER inhibitors over time (dt), $C_{HER} + S*C_{MEK}$ represents the normalized concentration of MEK and pan-HER inhibitors in the central compartment with a scaling factor (S) on MEK concentration, *f* is a linear function of $dA_{cumulative}$ with $B_0$ as intercept and $B_1$ as the slope of the exposure-toxicity relationship. $P_{DLT}$ is the probability of DLT as a function of logit-transformed *f*, in line with common probability estimations[28]. Also linear and log transformed estimations were used, but these resulted in unsuccessful minimizations and poor parameter precision.

Estimation of the first-order elimination rate from the effect compartment (recovery rate, $K_r$) resulted in very low estimates. Therefore, the elimination from the effect compartment was omitted from the model and the effect compartment can be interpreted as normalized cumulative exposure.

### Contribution of MEK and pan-HER inhibition to toxicity

Parameter estimates and the corresponding relationship between the amount in the effect compartment and the probability of DLT assuming equal contribution of MEK and HER inhibitors are shown in table 1 and figure 3.

**Table 1.** Parameter estimates for the combined model of MEK and pan-HER inhibitors.

| Parameters | Estimate | RSE (%) |
|---|---|---|
| Intercept ($B_0$) | -2.59 | 9 |
| Slope ($B_1$) | 0.214 | 31 |

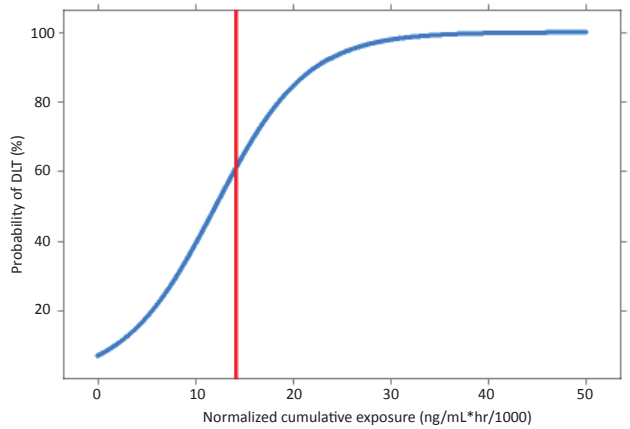


**Figure 3.** Relationship between the normalized cumulative exposure (ng/mL*hr/1000) and the predicted probability of dose-limiting toxicity (DLT) for the combined model with the maximum observed normalized cumulative exposure indicated by the red line.

A steep exposure-toxicity relationship with a sharp increase in the probability of DLT already at low exposures was identified, which is in accordance with the clinical observation of occurrence of DLT already at low dose levels. The normalized cumulative exposure in the clinical studies was on average 370 ng/mL*hr [range 0-14.000]. At this average observed normalized cumulative exposure, the estimated probability of DLT was 8%, whereas at the maximum observed normalized cumulative exposure, the estimated probability of DLT was 60%.

To assess the relative contributions of MEK and pan-HER inhibitors, a scaling factor (S) was applied to the normalized MEK concentrations. This scaling factor could not be accurately estimated. Therefore, a sensitivity analysis was conducted in which S was manually adapted with values between 0.1 to 10, which were considered the limits of a realistic scaling factor (i.e. a scale of 10 implies that the HER inhibitor affects the probability of DLT only by 10%, which is less than expected given that MEK and HER inhibitors both have substantial toxicities as a single agent). Within this range, likelihood profiling did not show a substantial OFV change (maximal difference +0.89) and parameter precision decreased.

Figure 4 illustrates the normalized cumulative exposure with S=1 per week of treatment and the corresponding predicted probability for DLT as well as the observed incidence of DLTs. With an increase of the normalized cumulative exposure, also the incidence of DLT increased from 0% in the first week of treatment (week 1), to 9% in week 2, and 19% in week 3 followed by a slight decrease in week 4 to 14%. This corresponded with an increasing maximum predicted probability per week of treatment from 10% in week 1 to 21% in week 2, 43% in week 3 and 59% in week 4.

Subsequently, the scaling factor S was estimated separately for the different studies. For the afatinib-selumetinib combination, a value of 1 was retained because values between 0.1 and 10 did not relevantly change the OFV (maximum dOFV +0.56) and parameter estimates. For the lapatinib-trametinib combination, a scaling factor of 10 improved the OFV (dOFV -5.5) although not significantly, whereas scaling by 0.1 resulted in a negligible OFV increase (dOFV +0.96). For the dacomitinib-PD0325901 combination, parameter precision was better with a scaling factor of 0.1 although the OFV remained unchanged. Altogether, also for the individual studies, the relative contribution of each compound could not be identified and the assumption of equal contribution of each compound was maintained.

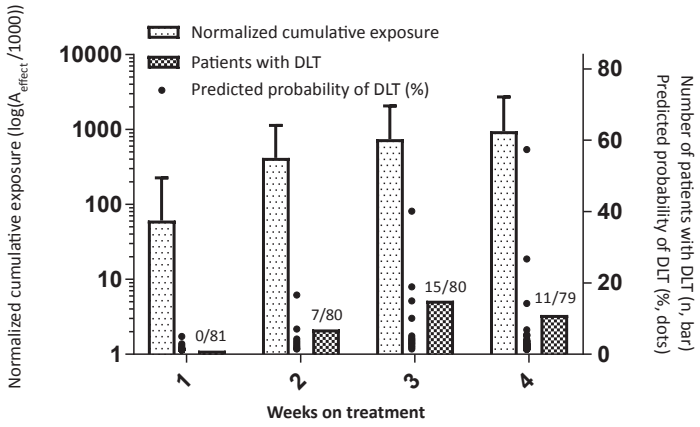


**Figure 4.** Normalized cumulative exposure to MEK and pan-HER inhibitors per week of treatment with the corresponding incidence of DLT (n (bars)) and the predicted probability of DLT (% (dots)).

## DISCUSSION

The relationship between exposure and toxicity was described by a sequential PK-TOX model which related the cumulative exposure of MEK and pan-HER inhibitors to the probability of DLT using a logistic regression model with logit transformation. This model was applied to a combined dataset for MEK and pan-HER inhibitors and to three separate models for the combinations of afatinib-selumetinib, lapatinib-trametinib and dacomitinib-PD-0325901.

With this model, we provide a novel approach to combine pharmacokinetic and toxicodynamic data from different clinical studies that investigate the same concept. We used a unique set of extensive PK and toxicity data from three ongoing clinical trials. It was considered rational to merge the PK and toxicity data from three trials based on the known overlap in toxicities between the different MEK inhibitors (selumetinib, PD-0325901 and trametinib[16,20,21]) and HER inhibitors (afatinib, dacomitinib, lapatinib[12,15,17]), i.e. class-related toxicities, and on the comparable trial design, data collection and data handling procedures. Dose finding in all three studies, according to a rule-based design, proved to be complicated due to occurrence of toxicity at relatively low dose levels. The nature of DLTs in the different studies were similar and consisted mainly of diarrhea, nausea, skin toxicity, liver damage and dehydration. Taken together, it was considered a rational approach to combine data to be able to quantify the relationship between drug exposure and toxicity and to assess contribution of drug class and/or individual drug on toxicity. Ultimately, this may assist in rational dose finding of this combination.

To enable merging of the data, the exposure to the different drugs was normalized on their reported $C_{trough}$. For example, median plasma concentrations for the MEK inhibitor selumetinib are around 1400 ng/ml (3.1 μM) dosed at the recommended single agent dose[21], whereas for trametinib concentrations reach only 22 ng/ml (0.031 μM)[16]. This normalization factor accounts for differences in plasma concentrations as a result of substance specific PK and expected concentrations at the recommended dose as single agents.

Importantly, there may be a discrepancy between the plasma levels and the tissue levels. In particular, the HER inhibitors have a large volume of distribution (Appendix 1) which may mean that more drug has distributed to the tissues with the potential of inducing damage. This is not reflected by our exposure-toxicity model and may lead to an underestimation of the effects of HER inhibition. Furthermore, this model does not differentiate between free and protein-bound drugs. Plasma concentrations were generated from the PK model which is based on measured total (bound and unbound) drugs. Although all drugs have extensive protein binding (in the range of >95%-99.9%) it is unsure if the use of total drug concentrations affects the ability of the model to discriminate between the effects of MEK and HER inhibitors.

A scaling factor was used to discriminate between the influence of drug class on probability of DLT. Other descriptive PK-TOX models for dual combinations have been described[29,30], but the distinction between effects of two classes of drugs has not been addressed earlier. Our baseline hypothesis was that MEK and HER inhibitors

have equal contributions to the probability of DLT (S=1), which was included in the initial model. Since this scaling factor could not be estimated, this hypothesis was tested by manual adaption of the scaling factor ($0.1 \leq S \leq 10$). However, none of the applied scales improved the model and we could not reject the initial hypothesis. Hence, class-specific contributions of MEK and HER inhibitors to the probability of DLT could not be identified.

Clinical implementation of these findings requires some cautions[31]. First of all, DLTs were modelled as binary outcomes while in practice this is a gradual process. However, the binary approach is in line with clinical trial practice, as it guides dose finding in the rule-based design of the studies. Although the model could be refined by defining toxicity as any toxicity that results in clinical interventions or treatment interruptions, for the scope of this study we aimed to select the most relevant toxicities by using DLTs as primary toxicity parameter. Secondly, the external validity of this model is unsure since only our dataset was available to use. Emerging data from the ongoing trials may help in establishing the robustness of the model. To test external validity, also data from other trials should be incorporated if feasible in the future.

The ultimate goal of this model was to develop an algorithm that could explain how MEK or pan-HER inhibition relates to toxicity to assist dose finding in combination trials. In the combined PK-TOX model, no differential effect of MEK or pan-HER inhibitors could be identified. This implies that dose-modifications of MEK and pan-HER inhibitors have similar impact. Also in the individual PK-TOX models for the three different combinations, no differential effects of the MEK and pan-HER inhibitors could be found. This suggests that a class-specific impact on toxicity of MEK or pan-HER inhibitors seems unlikely based on this model.

Our model confirmed and quantified our clinical finding that the dose range in which MEK and pan-HER inhibitors can be safely combined is narrow. Generally, a maximum probability of DLT of 33% is accepted for phase I clinical trials[32]. Although this incidence was not exceeded in the three clinical trials, our simulations show that the probability of 33% is already reached at relatively low exposures in the different clinical studies. This may also be a consequence of the fact that the dose-escalation started at 20-50% of the recommended single agent doses, and no information on lower dose-levels could be included. A better prediction of the dose-toxicity relationship could be obtained by including data from trials with these combinations in lower doses.

To conclude, based on our findings, the probability of DLT is dependent on the specific MEK-HER combination rather than on the extent of MEK or HER inhibition in general. Hence, we could not provide an algorithm to guide MEK or HER dosing in relation to DLTs. Without an algorithm, decisions on dose modifications in the clinic can only be made on an individual basis, dependent on the individual dose, drug and combination. In view of efficacy it should be taken into account that inhibition of MEK forms the basis of the responsive upregulation of HER proteins[5]. Herefore, it seems best to optimize MEK inhibition with a plasma concentration that exceeds the $IC_{50}$ or half maximal effective concentration ($EC_{50}$). Hence, reduction of HER inhibitors is preferred, and reduction of MEK can be considered if effective plasma concentrations can be maintained at the reduced dose. Hereby, it is most likely that the efficacy is preserved with reduced toxicity.

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Our gratefulness goes out to all patients who participated in the clinical studies.

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**APPENDIX 1 -** Output table pharmacokinetic models.

| Structural model parameters (fixed effects) [unit] | | Estimate | RSE% | Estimate |
|---|---|---|---|---|
| Lapatinib | | Theta | | Eta |
| Ke | [$h^{-1}$] | 0.0532 | 15% | 0.31 |
| Vd | [L] | 603 | 14% | - |
| Ka | [$h^{-1}$] | 2.59 | 7% | - |
| K12 | [$h^{-1}$] | 0.0756 | 36% | - |
| K21 | [$h^{-1}$] | 0.096 | 22% | - |
| Trametinib | | Theta | | |
| Ke | [$h^{-1}$] | 0.0189 | 19% | 0.32 |
| Vd | [L] | 230 | 19% | - |
| Ka | [$h^{-1}$] | 2.16 | 14% | - |
| K12 | [$h^{-1}$] | 0.709 | 22% | - |
| K21 | [$h^{-1}$] | 0.151 | 10% | - |
| Dacomitinib | | Theta | | |
| Ke | [$h^{-1}$] | 0.0223 | 48% | 0.237 |
| Vd | [L] | 1260 | 48% | - |
| Ka | [$h^{-1}$] | 0.585 | 25% | - |
| K12 | [$h^{-1}$] | 0.192 | 119% | - |
| K21 | [$h^{-1}$] | 0.148 | 48% | - |
| ERR1 | % | 3.88 | 71% | - |
| ERR2 | ng/mL | 0.277 | 9% | - |
| PD-0325901 | | Theta | | |
| Ke | [$h^{-1}$] | 0.217 | - | 0.343 |
| Vd | [L] | 19.5 | - | - |
| Ka | [$h^{-1}$] | 5.33 | - | - |
| K12 | [$h^{-1}$] | 0.601 | - | - |
| K21 | [$h^{-1}$] | 0.143 | - | - |
| K13 | [$h^{-1}$] | 0.06 | - | - |
| K31 | [$h^{-1}$] | 0.0008 | - | - |
| Afatinib | Parameters were fixed based on literature model [Freiwald *et al.* 2014 (1)] | Theta | | |
| Cl | [L*$h^{-1}$] | 42.3 | - | - |
| Vd | [L] | 456 | - | - |
| Ka | [$h^{-1}$] | 0.252 | - | 0.432 |
| K23 | [$h^{-1}$] | 0.17 | - | - |
| K32 | [$h^{-1}$] | 0.0685 | - | - |
| ALAG | [h] | 0 | - | - |
| F1 | - | 1 | - | 0.179 |
| Weight on CL | Weight fixed to: 75 kg | 0.595 | - | - |
| Creatinin on CL | Creatinin clearance fixed to: 79 ml/min | 0.0048 | - | - |
| Female | | 0.871 | - | - |
| Weight on Vd | Weight fixed to: 75 kg | 0.899 | - | - |
| Dose <70 mg | YES | 0.485 | - | - |
| Selumetinib | Parameters were fixed based on literature model [Patel *et al.* 2017 (2)] | Theta | | Eta |
| Cl | [L*$h^{-1}$] | 13.5 | | 0.0700 |
| Vd (2) | [L] | 32.6 | | 0.201 |
| Ka | [$h^{-1}$] | 3.7 | | - |
| K23 | [$h^{-1}$] | 0.252 | | 0.295 |
| K32 | [$h^{-1}$] | 0.149 | | 0.295 |
| ALAG1 | [h] | 0.319 | | 0.165 |
| F1 | - | 1 | | - |
| D1 | [$h^{-1}$] | 0.622 | | 0.171 |
| Vd (3) | [L] | 55 | | 0.388 |
| BSA on CL | BSA mean 1.95 | 0.924 | | - |
| ALT on CL | ALT fixed to: 20 | 0.187 | | - |
| BSA on Vd (2) | BSA mean:1.95 | 1.24 | | - |
| Age on Vd (2) | Age mean: 65 | 0.327 | | - |

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**APPENDIX 2 -** Overview of literature ($C_{trough}$) based normalization levels for each drug.

| Agent | Normalization level (ng/mL) | Reference |
|---|---|---|
| Afatinib | 20 | Wind [1] |
| Dacomitinib | 71.3 | Takahashi [2] |
| Lapatinib | 300 | Burris [3] |
| PD-0325901 | 63.4 | LoRusso [4] |
| Selumetinib | 400 | O'Neill [5] |
| Trametinib | 12.1 | Infante [6] |

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# Chapter 3

# Phase I studies with biological targeted anticancer agents

## Chapter 3.1

**$^{89}$Zr-labeled CEA-targeted IL-2 variant immunocytokine in patients with solid tumors: role of IL-2 receptor-binding and CEA-mediated tumor accumulation**

*Submitted for publication*

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**ABSTRACT**

**Background** Cergutuzumab amunaleukin (CEA-IL2v) is an immunocytokine directed against Carcinoembryonic Antigen (CEA) containing an IL2v-moiety with abolished IL-2 receptor (IL-2R) α (CD25) binding, designed to improve IL-2 therapy. We describe biodistribution and tumor accumulation of $^{89}$Zr-labeled CEA-IL2v.

**Methods** Patients with advanced solid CEA positive (CEA+) or negative (CEA-) tumors were treated biweekly with 6, 20 or 30 mg CEA-IL2v. At cycle 1 50 MBq/2 mg $^{89}$Zr-CEA-IL2v was administered <1h after dosing followed by up to three $^{89}$Zr-PET assessments (day 1, 2, 5, 9). Patients with visually confirmed $^{89}$Zr-CEA-IL2v tumor accumulation at 20 mg had repeated $^{89}$Zr-PET imaging during treatment in cycle 4.

**Results** At 6 mg (4 CEA+; 3 CEA-), 20 mg (9 CEA+) and 30 mg (4 CEA+; 4 CEA-) CEA-IL2v, highest accumulation of $^{89}$Zr-CEA-IL2v was observed on day 5 in spleen (% injected dose [%ID]/$mL_{mean}$ $1.2 \cdot 10^{-2} \pm 0.42 \cdot 10^{-2}$) and liver (%ID/$mL_{mean}$ $1.1 \cdot 10^{-2} \pm 0.35 \cdot 10^{-2}$) generally independent of dose. In all cohorts, accumulation in non-pathological lymph nodes was observed. Tumor accumulation of $^{89}$Zr-CEA-IL2v was observed in all CEA+ cohorts: 3/4 evaluable patients at 6 mg, 7/8 at 20 mg and 4/4 at 30 mg in cycle 1. In CEA- tumors, tumor accumulation was seen at 30 mg only. In cycle 4, accumulation decreased by 57%, possibly due to peripheral IL-2R expansion.

**Conclusion** $^{89}$Zr-CEA-IL2v immuno-PET demonstrated generally dose- and CEA-independent biodistribution during treatment with major accumulation in lymphoid tissue. Tumor accumulation of $^{89}$Zr-CEA-IL2v was CEA-mediated with consistent accumulation at doses ≥20 mg supporting dose and schedule selection for future clinical applications.

## INTRODUCTION

Suppression of the immune system has been recognized as an emerging hallmark of many types of cancer. Indeed, stimulation of the immune system has proven to induce significant anti-tumor immune responses in several types of cancer including non-small cell lung, head and neck, renal cell, colorectal cancer and melanoma[1,2]. Since the early 90s immuno-stimulation with interleukin-2 (IL-2) has been applied for treatment of melanoma and renal cell carcinomas[3]. However, application of IL-2 has been limited by its short half-life and severe toxicities such as vascular-leak syndrome, acute respiratory disorders, and hypotension[4]. Additionally, IL-2 activates regulatory T cells ($T_{regs}$), which has an immunosuppressive effect. Both activation of $T_{regs}$ and (pulmonary) vascular leakage are considered to be mediated by binding of IL-2 to IL-2-receptor alpha (IL-2Rα/CD25)[5]. This high-affinity receptor subunit is preferentially expressed on $T_{regs}$ and endothelial cells but it is not required for effector T-cell activation and expansion, which occurs primarily through the IL-2Rβ and IL-2Rγ subunits[6].

To improve existing IL-2 therapy, the targeted immunocytokine cergutuzumab amunaleukin (CEA-IL2v) has been designed. CEA-IL2v consists of a carcinoembryonic antigen (CEA)-targeted $IgG_1$-antibody fused to an engineered IL-2 variant (IL2v) with abolished IL-2Rα binding. Its molecular design aims to induce a local immune response in the tumor by binding preferentially to CEA-expressing tumor cells and to avoid activation of $T_{regs}$ due to reduced IL-2Rα binding, which was confirmed in preclinical experiments[7]. CEA is an attractive target for anticancer therapy because of its widespread expression on tumors. CEA is normally expressed during embryonal development and on healthy colon mucosa and is present as soluble CEA in the circulation. Nearly all colorectal cancers, gastric cancers and pancreatic cancers overexpress CEA as well as 70% of the non-small cell lung cancers and 50% of breast cancers[8,9]. To avoid trapping to soluble CEA, CEA-IL2v contains a membrane-proximal CEA target epitope which does not recognize soluble CEA[7]. Taken together, the characteristics of CEA-IL2v support its potential to achieve a stronger activity on immune effector cells than on $T_{regs}$, a more favorable pharmacokinetic and biodistribution profile and better tolerability compared to existing IL-2 therapy.

In order to confirm the supposed dual binding to CEA and IL-2R, an immuno positron emission tomography (PET) substudy was performed as part of the BP28920 first-in-man phase I trial with CEA-IL2v (NCT02004106). Immuno-PET using Zirconium-89 ($^{89}$Zr) labeled mAbs provides a non-invasive tool for *in vivo* visualization and quantification of mAbs[10]. We report biodistribution and tumor accumulation of $^{89}$Zr-CEA-IL2v, in relation to CEA status of the tumor and treatment dose. Furthermore, we discuss the added value of on-treatment imaging based on observed alterations in biodistribution during treatment in this trial.

## METHODS

### Patient population

Patients with advanced and/or metastatic solid tumors without standard treatment options were included if they had CEA positive (CEA+) tumors defined as ≥20% of tumor cells with moderately intense staining or, as a control group, CEA- defined as 0% staining by immunohistochemistry on archival or freshly obtained tumor material. Staining was performed locally with a CEA31 mouse monoclonal $IgG_1$ anti CD66/CEACAM5 antibody (Cell Marque #760-4594, Ventana Medical Systems, USA) using an in-house validated procedure. Other eligibility requirements were age ≥18 years, ≥ one tumor lesion accessible for a biopsy, ≥ one non-liver lesion assessable by $^{89}$Zr-PET imaging and radiologically measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, life expectancy of more than 12 weeks, World Health Organization (WHO)/ Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate organ function including hematological, renal, hepatic, and coagulation parameters. Exclusion criteria included history of or current central nervous system tumors, active second malignancies, except non-melanoma skin cancer or cervical carcinoma *in situ*, active infections, uncontrolled concomitant diseases or mental illnesses which could affect protocol compliance, uncontrolled hypertension, pregnancy, human immunodeficiency virus, major surgery and/or immunotherapies or immunosuppressive drugs within 28 days prior to start, hypersensitivity to the investigational drug, premedications (corticosteroids, antihistamines, paracetamol or 5-$HT_3$ antagonists) or 2-($^{18}$F)-fluoro-2-deoxyglucose, concurrent therapy with investigational drugs, chronic or high-dose use of corticosteroids (>20 mg dexamethasone-equivalents), immunosuppressive therapy, baseline QTc interval >470 ms, bradycardia (<45 bpm) or tachycardia (>100 bpm) and wide-field radiotherapy within four weeks prior to start.

### Study design

This study was executed in VU University Medical Center (VUmc) Cancer Center and the Netherlands Cancer Institute in Amsterdam, the Netherlands, as a substudy of the first-in-man trial with CEA-IL2v (NCT02004106). The

study protocol and all amendments were approved by the local ethics committees. All patients provided written informed consent before any study procedure. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guideline with the ethical principles of the current version of Declaration of Helsinki and local regulatory guidelines.

**Study procedures**

CEA-IL2v was administered intravenously biweekly in two hours to patients with CEA+ tumors (cohort A: 6 mg, cohort C: 30 mg and 20 mg for the last patient) and CEA negative (CEA-) tumors (cohort B: 6 mg, cohort D; 30 mg) (Supplemental Material Figure 1). Based on emerging safety data from the phase I trial, the last patient in cohort C received 20 mg and a subsequent cohort E received 20 mg in cycle 1 and 30 mg in cycle 2 and onwards. For PET imaging, CEA-IL2v was labeled at VUmc with $^{89}$Zr (BV Cyclotron VU, Amsterdam, the Netherlands) according to Good Manufacturing Practice (GMP) standards, as previously described[11]. In cycle 1 on day 1, 2 mg /50 MBq $^{89}$Zr-CEA-IL2v was infused in 10 minutes within 1 hour after infusion of unlabeled CEA-IL2v as part of the total CEA-IL2v dose. Whole body PET-low dose computed tomography (ldCT) and $^{89}$Zr-PET scans were acquired on a Gemini TF-64 or Ingenuity TF PET/CT scanner (Philips Healthcare, Best, the Netherlands) and scheduled on day 1 (2 hours post injection [p.i.]) (for dosimetry purposes in the first three patients only), day 2 (24 hours p.i.), day 5 (96 hours p.i.) and day 9 (192 hours p.i.) with a maximum of three PET scans per patient after tracer administration. Scanners were EARL accredited, cross-calibrated and images were reconstructed as previously described[12]. $^{18}$F-FDG-PET scans were performed at baseline according to European Association of Nuclear Medicine (EANM) 2.0 guidelines[13] to identify evaluable malignant lesions for $^{89}$Zr-PET imaging. After having completed the imaging procedures in cycle 1, patients continued in the first-in-human study and received CEA-IL2v in the highest dose cohort that was cleared for safety.

In cohort E, patients with confirmed $^{89}$Zr-CEA-IL2v tumor accumulation by visual analysis underwent an additional administration of 2 mg/50 MBq $^{89}$Zr-CEA-IL2v <1 hour after administration of unlabeled CEA-IL2v in treatment cycle 4 with subsequent $^{89}$Zr-PET scans at day 2 and 5. Patients continued treatment until confirmed disease progression, death, unacceptable toxicity, withdrawal of consent, or investigators' decision to stop. Response assessment was done according to RECIST 1.1 by CT at baseline and at 12 weeks, and every eight weeks thereafter. For $^{89}$Zr-CEA-IL2v pharmacokinetic and CEA-IL2v pharmacodynamic analyses, whole blood and serum was collected via an intravenous catheter at cycle 1 day 1 predose, end of infusion, 2 hours, 4 hours p.i. and at day 2, 5 and 9. For patients receiving a second $^{89}$Zr-CEA-IL2v administration in cycle 4, samples were collected at the same timepoints in cycle 4. Laboratory checks and safety assessments were done throughout the study.

**$^{89}$Zr-CEA-IL2v PET data analysis**

Visual assessment of biodistribution and tumor accumulation was performed by three (technical) physicians experienced in PET image analysis (OSH/LWV/MST): initially, assessments of $^{89}$Zr-PET alone was done to identify positive lesions, followed by combined assessment with $^{18}$F-FDG-PET and clinical history to confirm $^{89}$Zr-positive lesions as tumor lesions or non-pathological lesions, and to identify $^{89}$Zr-negative lesions. Reading was performed by two teams independently and in case of disagreement reading was repeated to obtain consensus. Tumor accumulation of $^{89}$Zr-CEA-IL2v was defined as visually enhanced accumulation exceeding local background. At a patient level, $^{89}$Zr-PET scans were considered positive if at least one non-nodal tumor lesion showed $^{89}$Zr accumulation on the cycle 1 day 5 scan and one additional scan supporting consistent tumor accumulation. Quantification of ($^{18}$F-FDG positive) tumor lesions was performed for all timepoints. Quantitative evaluation of tumor accumulation was based on the day 5 scan in $^{89}$Zr-CEA-IL2v positive tumor lesions ≥10 mm and lymph nodes ≥15 mm according to RECIST 1.1 criteria for measurable lesions, to reduce the contribution of partial volume effects. Smaller lesions were considered not evaluable for quantification. Visually $^{89}$Zr- CEA-IL2v positive non-pathological ($^{18}$F-FDG negative) lymph nodes were analyzed visually only as quantification of lesions <15 mm is not reliable due to partial volume effects.

In all $^{89}$Zr-PET scans, volumes of interest (VOIs) of whole organs liver, spleen, kidney, lung and bone marrow and of tumor lesions were manually delineated to derive percentage of injected dose per volume of tissue of interest (%ID/mL) as $\%ID/mL_{mean}$ for tissue and $\%ID/mL_{peak}$ for tumor to account for segmentation errors and background accumulation using in-house developed software[12]. For lung, VOIs were semi-automatically defined on the ldCT and projected on the PET images. VOIs of the liver, spleen and kidney were manually delineated on the PET images themselves using the ldCT as reference. Fixed sized VOIs with volumes of 8.6 mL were placed on lumbar vertebrae on ldCT. Serum and whole blood $^{89}$Zr-CEA-IL2v concentrations were assessed by radioactivity measurements in a cross-calibrated gamma counter (Wizard 3, PerkinElmer, USA in the Netherlands Cancer Institute and Wallac Wizard 1480, PerkinElmer, USA in VUmc).

**Patient data**

Baseline characteristics were collected including age, gender, Eastern Cooperative Oncology Group (ECOG) performance status and tumor type. Serum drug (unlabeled CEA-IL2v) and anti-CEA-IL2v antibody concentrations were determined at a central laboratory by a validated ELISA method. The assay to detect serum drug used a biotinylated mouse monoclonal antibody directed against the idiotype of CEA-IL2v as capture reagent and digoxigenylated recombinant human IL-2R along with horseradish peroxidase conjugated anti-digoxigenin fragments as detection molecules. This bi-functional, target-binding competent assay has a sensitivity of 0.7 ng/mL in human serum. The bioanalytical method for the detection of anti-CEA-IL2v antibodies has been described previously[14]. Briefly, a bridging ELISA was used with biotinylated and digoxigenylated CEA-IL2v as capture/detection reagents in a three-tiered approach starting with ADA screening (tier 1) and following confirmation (tier 2) and titration assay (tier 3). Affinity-purified anti-idiotypic polyclonal antibodies directed against CEA-IL2v were used as a positive control. The sensitivity of the ELISA is 15.1 ng/mL for the used positive control.
Effects of CEA-IL2v on immune cell subsets were determined by lymphocyte counts ($CD^{3+}$, $CD^{4+}$, $CD^{8+}$, $CD^{16+}$, $CD^{56+}$, $FoxP3^{+}$) by fluorescence-activated cell sorting (FACS) in peripheral plasma. Safety was assessed by Common Terminology Criteria for Adverse Events (CTCAE) v4.03 although the full safety profile was the objective of the separate phase I study and toxicities are not extensively described in this paper.

**Statistical analysis**

Since the objectives of this study are purely descriptive, no formal statistical justification is provided for the sample size. Patients were evaluable for biodistribution imaging if they had at least one $^{89}$Zr-PET scan after administration of $^{89}$Zr-CEA-IL2v and also for tumor accumulation if at least one $^{18}$F-FDG positive (+) tumor lesion was $^{89}$Zr-PET assessable. Patients were evaluable for safety and pharmacokinetics after administration of one dose of (unlabeled) CEA-IL2v. For efficacy analysis one on-treatment tumor assessment was required. The relationship between organ accumulation, dose and CEA status was analyzed by One Way Analysis of Variance (ANOVA). The difference between organ accumulation in cycle 1 and cycle 4 per patient was analyzed by a two-tailed paired Students'T-test. All analyses were performed in R[15], software for statistical computing and graphics.

**RESULTS**

**Patients and treatment**

Between June 2014 and March 2016, 24 patients were enrolled. Primary CEA+ (n=17) tumors included colorectal cancer (n=11), non-small cell lung cancer (n=4), salivary gland cancer (n=1) and gastric cancer (n=1). CEA- tumors (n=7) were renal cell cancer (n=3), melanoma (n=2) pancreatic cancer (n=1) and ovarian cancer (n=1). Baseline patient characteristics are summarized in Supplemental Material Table 1. Patients were treated in all cycles with 6 mg (cohort A; CEA+ n=4, cohort B; CEA- n=3), 30 mg (n=8) (cohort C; CEA+ n=4, cohort D; CEA- n=4), 20 mg (cohort C; CEA+ n=1) or 20 mg in cycle 1 and 30 mg in cycle 2 and onwards (Cohort E; CEA+ n=8). One patient in cohort E was not evaluable for tumor accumulation due to lack of $^{89}$Zr-PET evaluable tumor lesions but normal tissue accumulation was evaluable for biodistribution analyses. All patients underwent at least one $^{89}$Zr-CEA-IL2v administration and subsequent PET imaging at start of treatment. Of the eight patients treated with 20 mg in cycle 1 and evaluable for tumor accumulation, four out of six patients with initial tumor accumulation had a second $^{89}$Zr-CEA-IL2v administration and underwent $^{89}$Zr-PET imaging on treatment in cycle 4.

The most frequently observed adverse events related to treatment with CEA-IL2v were infusion related reactions (63%), pyrexia (54%), fatigue and nausea (46% for both). Four patients discontinued treatment due to adverse events (pain, dyspnea, pulmonary hypertension and diarrhea). No adverse events specifically due to $^{89}$Zr-CEA-IL2v have been observed.

**Biodistribution**

$^{89}$Zr-CEA-IL2v blood levels and biodistribution in cycle 1 are presented in figures 1 and 2. Immediately after infusion (t=0), the mean recovered dose of $^{89}$Zr-CEA-IL2v in whole blood was 19%/L [range: 8-31%/L] and 33%/L [range: 17-53%/L] in serum. $^{89}$Zr -CEA-IL2v was cleared from the blood in a dose-independent manner with an apparent elimination half-life of 34 [range: 9-53] hours independent of dose. Pharmacokinetic curves of $^{89}$Zr-CEA-IL2v showed similar patterns to those of unlabeled CEA-IL2v yet the peak concentrations on the first day of infusion were less pronounced for unlabeled CEA-IL2v (Supplemental Material Figure 2). Anti-CEA-IL2v anti-drug antibodies (ADAs) were detected in 14 out of 20 patients with evaluable samples (70%), in most ADA positive patients already at the first assessment (42 days).

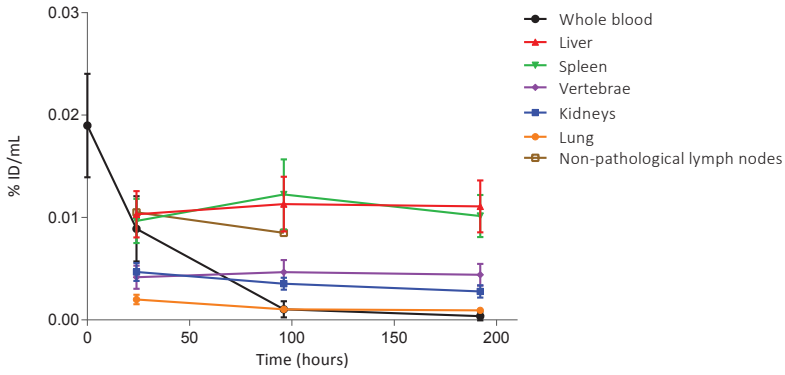


**Figure 1.** Distribution of $^{89}$Zr-CEA-IL2v as percentage of injected dose per milliliter (%ID/mL) over time in cycle 1 assessed on day 2, 5 and 9; organ uptake as %ID/mL mean, whole blood as %ID/mL and non-pathological lymph nodes that met the criteria for quantification as %ID/mL peak.

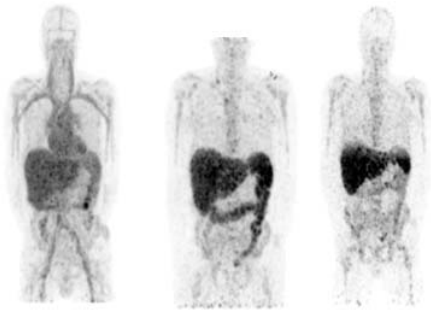

**Figure 2.** Biodistribution on day 2, 5 and 9. Representative maximum intensity projection of a CEA- renal cell carcinoma patient treated with 30 mg $^{89}$Zr –CEA-IL2v showing hepatobiliary excretion on day 5.

On day 2, tissue accumulation was highest in the spleen (%ID/mL$_{mean}$ 0.97 $\cdot 10^{-2} \pm 0.022 \cdot 10^{-2}$) and in the liver (%ID/mL$_{mean}$ 1.0 $\cdot 10^{-2} \pm 0.23 \cdot 10^{-2}$), which remained high at day 5 (%ID/mL$_{mean}$ 1.2 $\cdot 10^{-2} \pm 0.42 \cdot 10^{-2}$, 1.1 $\cdot 10^{-2} \pm 0.35 \cdot 10^{-2}$) and day 9 (%ID/mL$_{mean}$ 0.97 $\cdot 10^{-2} \pm 0.35 \cdot 10^{-2}$, 1.0 $\cdot 10^{-2} \pm 0.39 \cdot 10^{-2}$) (figure 1). Lower uptake was found in the kidney, lung and vertebrae (day 2 %ID/mL$_{mean}$ 0. 47$\cdot 10^{-2} \pm 0.89 \cdot 10^{-3}$, 0.19 $\cdot 10^{-2} \pm 0.61 \cdot 10^{-3}$ and 0.42$\cdot 10^{-2} \pm 0.11 \cdot 10^{-2}$ respectively). Uptake in these organs decreased slightly from day 5 (day 5 %ID/mL$_{mean}$ 0.34 $\cdot 10^{-2} \pm 0.92 \cdot 10^{-3}$, 0.10 $\cdot 10^{-2} \pm 0.31 \cdot 10^{-3}$ and 0.45 $\cdot 10^{-2} \pm 0.15 \cdot 10^{-2}$) to day 9 (day 9 %ID/mL$_{mean}$ 0.26 $\cdot 10^{-2} \pm 0.96 \cdot 10^{-3}$, 0.87 $\cdot 10^{-3} \pm 0.37 \cdot 10^{-3}$ and 0.41 $\cdot 10^{-2} \pm 0.16 \cdot 10^{-2}$). Elimination occurred via hepatobiliary excretion as can be seen on the day 5 $^{89}$Zr-PET scan (figure 2). Biodistribution was generally independent of dose and CEA status (Supplemental Material Table 2). Only splenic uptake was found to be significantly lower in the patients treated with 30 mg compared to the other dose-cohorts.

Also, enhanced $^{89}$Zr-accumulation was observed in non-pathological ($^{18}$F-FDG negative) lymph nodes in eight patients from different cohorts. Seventeen $^{89}$Zr-positive non-pathological lymph nodes were identified. As expected for non-pathological lymph nodes they were not evaluable for quantification due to small size (<15 mm).

**Tumor accumulation**

Of 24 patients included, 23 were evaluable for analysis of tumor accumulation with $^{89}$Zr-PET. One patient was excluded because the tumor was $^{18}$F-FDG-negative, which did not meet the criteria for evaluability. At visual image analysis, there was enhanced tracer accumulation in tumor lesions in 14 out of 16 patients with CEA+ tumors (88%) and in four out of seven patients with CEA- tumors (57%) (Supplemental Material Table 3). There was a trend towards more consistent tumor accumulation in CEA+ patients at higher doses (figure 3 and Supplemental Material Figure 3): in three out of four CEA+ patients treated at 6 mg (75%), seven out of eight

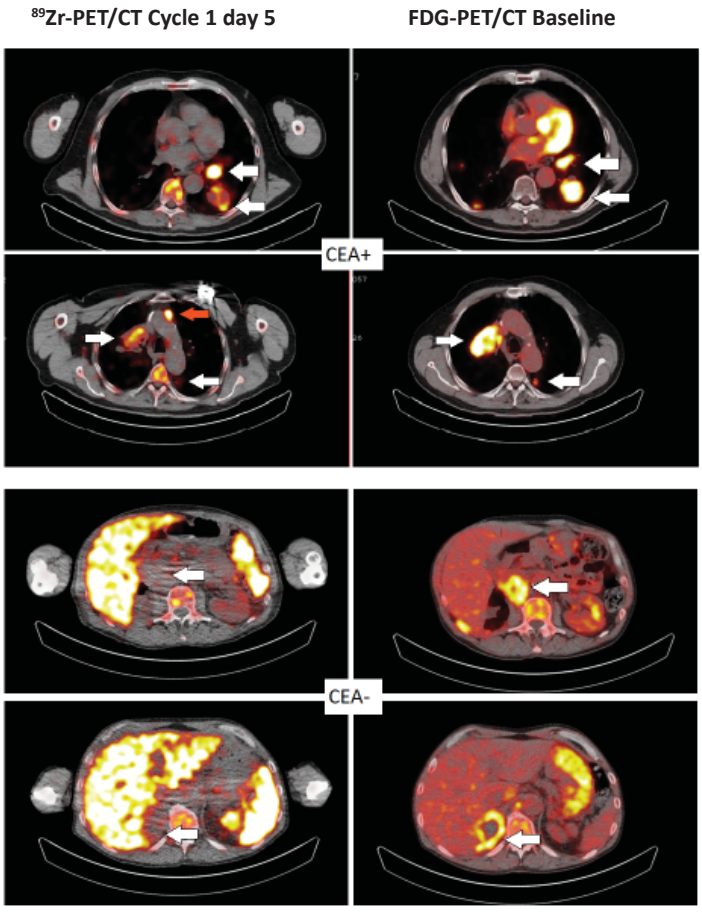


**Figure 3.** Tumor accumulation in a CEA+ and a CEA- patient treated with 20 mg and 30 mg, respectively. Images of $^{89}$Zr-PET at cycle 1 day 5 are shown (left) and tumor lesions were identified by $^{18}$F-FDG-PET at screening (right). Accumulation was observed in the left and right hilar lymph nodes, the left dorsal lung lesion (accumulation indicated by white arrows) and a non-pathological lymph node (accumulation indicated by red arrow) in the CEA$^{+}$ patient (upper panels). For the CEA$^{-}$ patient the adrenal gland and abdominal lymph node lesions were negative on $^{89}$Zr-PET (lower panels).

at 20 mg (88%) and four out of four at 30 mg (100%). No accumulation was observed in CEA- tumors in all three patients at doses of 6 mg, however at 30 mg tumor accumulation was seen in all patients, although to a lesser extent than in CEA+ tumor lesions (mean $\%ID/mL_{peak}$ CEA+ $6.7 \cdot 10^{-3} \pm 5.9 \cdot 10^{-3}$ vs. CEA- $3.6 \cdot 10^{-3} \pm 1.7 \cdot 10^{-3}$, $p = 0.15$). Tumor accumulation differed between the location of tumor lesions (figure 4): highest accumulation was found in bone metastases ($\%ID/mL_{peak}$ $9.1 \cdot 10^{-3} \pm 3.5 \cdot 10^{-3}$) and lymph nodes ($\%ID/mL_{peak}$ $7.2 \cdot 10^{-3} \pm 3.9 \cdot 10^{-3}$), and the lowest accumulation was found in pulmonary lesions ($\%ID/mL_{peak}$ $3.6 \cdot 10^{-3} \pm 2.2 \cdot 10^{-3}$). Accumulation in lesions located elsewhere, including the adrenal gland and soft tissue, was slightly higher than in lung lesions ($\%ID/mL_{peak}$ $6.2 \cdot 10^{-3} \pm 2.4 \cdot 10^{-3}$). Tumor lesions located in the colorectal tract and liver could not be accurately quantified due to high background accumulation with resulting spill-in effects. The majority of visually positive tumor lesions was positive at all timepoints. Five tumor lesions (three lymph nodes, one lung and one liver) showed visually enhanced accumulation on only one timepoint. Out of 21 patients evaluable for response, the best response was stable disease in three patients (14%) and progressive disease in 18 patients (86%). There was no relation between accumulation and response.

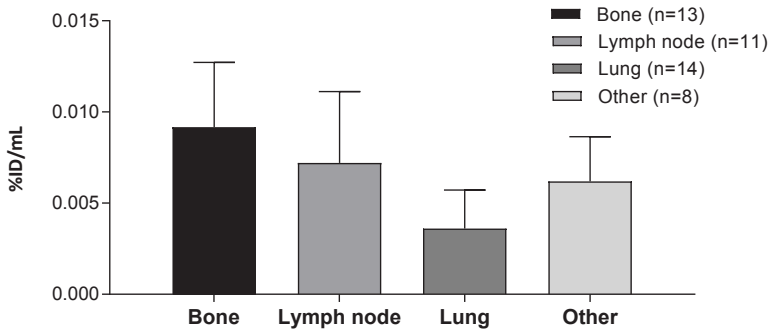


**Figure 4.** Tumor targeting at cycle 1 day 5 of different lesions from CEA$^+$ patients treated with doses ≥ 20 mg in peak percentage of injected dose per mL (% ID/mL$_{peak}$) with the corresponding number of lesions per group (n).

**Changes in biodistribution and tumor accumulation during treatment**

In cohort E, four patients underwent two administrations of $^{89}$Zr-CEA-IL2v, one before treatment in cycle 1 and one on-treatment in cycle 4. At cycle 4, a reduced exposure to $^{89}$Zr-CEA-IL2v was observed by a decreased area under the serum concentration-time curve (AUC cycle 4$_{0\text{-}192\ hours}$ vs. AUC cycle 1$_{0\text{-}192\ hours}$ -23%, data not shown). At the same time, tissue distribution (%ID/mL$_{mean}$) had changed with a 38% lower splenic accumulation ($p$=0.001), and 29% higher hepatic accumulation ($p$=0.02) than in cycle 1 (figure 5 and 6). Tracer accumulation in the vertebrae, kidney and lung did not change over time.

In cycle 4, tumor accumulation as defined with %ID/ml$_{peak}$ at day 5 was lower than in cycle 1: whereas in these four patients 15 lesions had visually confirmed accumulation in cycle 1, only nine lesions showed accumulation in cycle 4 and accumulation decreased by 57% (mean %ID/mL$_{peak}$ in the four patients in cycle 1; $7.3 \cdot 10^{-3} \pm 2.1 \cdot 10^{-3}$ vs. $3.1 \cdot 10^{-3} \pm 3.3 \cdot 10^{-3}$ in cycle 4, $p$ <0.01).

**DISCUSSION**

In this clinical immuno-PET study we showed CEA-mediated tumor accumulation of $^{89}$Zr-CEA-IL2v in patients with solid CEA-expressing tumors whereas only limited accumulation was observed in CEA- tumors. In addition, this study demonstrated that exposure to CEA-IL2v led to changes in biodistribution and tumor accumulation after multiple CEA-IL2v administrations.

Tumor accumulation was quantified on day 5 because image quality was optimal with the lowest background activity, which is comparable to previous clinical studies with $^{89}$Zr-labeled antibodies[10,16]. Only tumor lesions ≥10 mm (≥15 mm for lymph nodes) were quantified to limit partial volume effects to <50% of variability in the recovered activity[12]. The relatively high uptake of $^{89}$Zr-CEA-IL2v in bone lesions compared to other tumor locations may be related to the bone-seeking characteristic of $^{89}$Zr when released from the conjugate[17]. Based on the stability of the conjugation method[18] and product (Roche, unpublished internal data) we expect that limited free $^{89}$Zr is present in circulation, which is supported by the fact that no significant accumulation in healthy bone was observed. Alternatively, bone metastases may be more easily accessible for antibodies, which has been observed for other $^{89}$Zr-labeled antibodies too[19]. The high accumulation in pathological lymph nodes can be related to the accumulation seen in non-pathological lymph nodes, which may both be IL-2R mediated. As expected due to size limitations, accumulation in non-pathological lymph nodes could not reliably be quantified for comparison, and it stays unclear to what extent accumulation in pathological lymph nodes is tumor-specific or IL-2R mediated. At baseline, tumors were classified as CEA+ and CEA- based on archival material from the primary tumor or a metastasis. Tumor accumulation was higher and more consistent in CEA+ patients compared to CEA- patients (figure 3 and Supplemental Material Figure 3), supporting CEA-mediated tumor accumulation. CEA-mediated tumor accumulation has been reported before in several clinical trials with anti-CEA antibodies[16,20–25], mainly in colorectal, liver, bone, thyroid and lymph node lesions. In these trials, tumor accumulation was considered CEA-mediated, yet none of the trials confirmed tumor CEA expression with immunohistochemistry. For other bispecific CEA constructs consisting of anti-CEA-CD3 and anti-CEA-B7, CEA-mediated accumulation was confirmed as well, albeit only in preclinical experiments[26–28]. In our study, tumor accumulation was still present at day 9 while most of the drug was cleared from blood, confirming retention in the tumor. This retention was most pronounced in the cohort treated with the highest dose (30 mg). At 30 mg

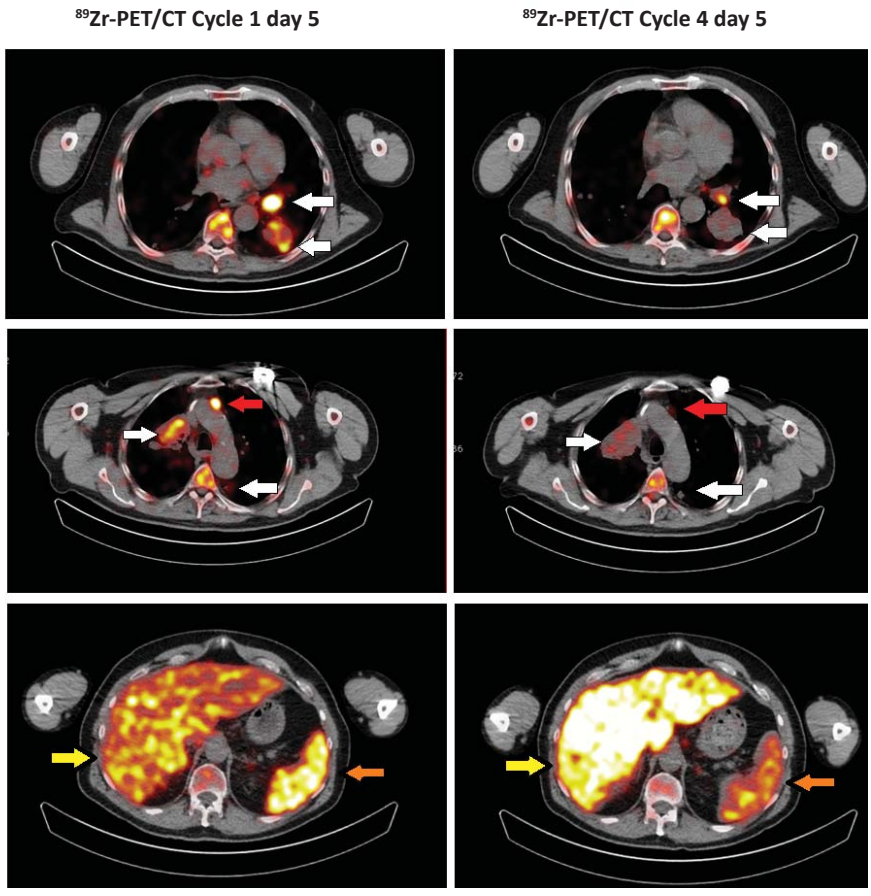


**Figure 5.** Tumor accumulation decreased between cycle 1 day 5 and cycle 4 day 5 (tumor lesions indicated by white arrows in the upper and middle panel, non-pathological lymph node indicated by a red arrow) as did spleen accumulation (orange arrows, lower panel). Liver accumulation was increased in cycle 4 (yellow arrows, lower panel). Evaluable lesions included left and right hilar lymph nodes and a left-sided dorsal lung lesion.

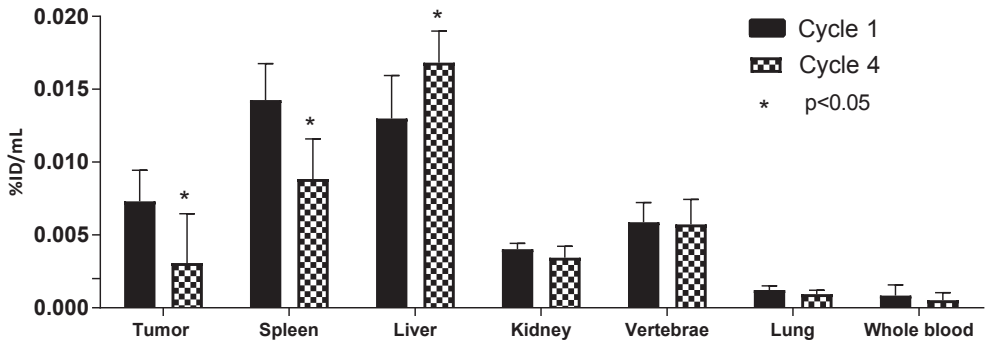


**Figure 6.** Tumor, organ and whole blood accumulation in cycle 1 day 5 versus cycle 4 day 5 as percentage of injected dose per mL (%ID/mL) (whole blood) or as %ID/mL $_{mean}$ (organs) or %ID/mL $_{peak}$ (tumor) (n=4). The asterisk (*) indicates a statistically significant difference, assessed by a two-tailed paired Students'T-test.

CEA-IL2v, also some accumulation was observed in CEA- tumors, albeit less than for CEA+ tumors. Although intratumoral heterogeneity of CEA expression is not well described in literature[29], it seems to be limited[30] making it highly unlikely that in CEA- tumors still a CEA-mediated accumulation occurs. Therefore, the tumor accumulation observed in CEA- tumors could be due to IL-2-mediated binding to tumor infiltrating lymphocytes, which was previously reported for IL-2 in melanoma, head and neck- and renal cell carcinoma as well[31–33]. In addition, at all doses, $^{89}$Zr-CEA-IL2v accumulation was high in lymphoid organs such as spleen and some non-pathological lymph nodes, supporting the supposed bispecific activity of this immunocytokine binding CEA as well as IL-2R.

Based on our data, the overall pharmacokinetic profile of $^{89}$Zr–CEA-IL2v is comparable to unlabeled CEA-IL2v. However, on day 1 shortly after infusion we observed relatively low levels of unlabeled CEA-IL2v whereas a pronounced $^{89}$Zr-CEA-IL2v peak concentration was present (Supplemental Material Figure 2). This may be due to a difference in sensitivity of the analytical methods; detection of $^{89}$Zr-CEA-IL2v is based on radioactivity measurements whereas unlabeled CEA-IL2v is detected by ELISA. Also, the one-hour interval between administration of unlabeled and labeled $^{89}$Zr-CEA-IL2v could result in discrepancies in pharmacokinetic profiles.

Like other antibodies, $^{89}$Zr-CEA-IL2v distributed to highly perfused tissues such as liver, spleen and bone marrow and to a lesser extent to lung, kidneys and lymph nodes[34]. In lung, kidney and bone marrow, CEA-IL2v accumulation seemed directly related to the blood concentration. Accumulation in tumor lesions, spleen and liver was considered related to specific binding and metabolism, respectively. Although lymph node accumulation is generally minor for antibodies[34], it was pronounced for $^{89}$Zr-CEA-IL2v both in pathological and non-pathological lymph nodes, likely IL-2R mediated[31–33]. Biodistribution was generally independent of dose and CEA status. Although based on our results, on preclinical pharmacokinetics of this compound[35] and on previous studies with anti-CEA antibodies[16], an increased clearance at higher doses and in CEA+ cohorts may be expected, this was not observed in our study. Possibly, this may be due to small cohort sizes and high intra-patient variability in pharmacokinetics[25,36].

Uniquely, this study incorporated on-treatment $^{89}$Zr-PET imaging in cycle 4 which enabled us to identify changes in tissue biodistribution over time. We found that liver accumulation was increased in cycle 4 with lower $^{89}$Zr-CEA-IL2v spleen and tumor accumulation and exposure (AUC). The observed AUC reduction may be due to expansion of IL-2R expressing cells increasing sequestering of CEA-IL2v (Supplemental Material Figure 4). In spite of lymphocyte expansion, spleen accumulation diminished in cycle 4. We hypothesize this may be a result of IL-2R occupancy with unlabeled CEA-IL2v leading to reduced IL-2R-bound $^{89}$Zr-CEA-IL2v. This mechanism could also explain the reduced spleen accumulation in the 30 mg cohort compared to other cohorts. The simultaneously increased liver accumulation in cycle 4 may be explained by increased $^{89}$Zr-CEA-IL2v metabolism in the liver. Liver metabolism could be enhanced by ADAs[37,38], which were found in 70% of the patients in this study. In addition, these ADAs could hinder binding to tumor cells or lymphocytes resulting in decreased spleen and tumor accumulation by shielding the IL-2v or the anti-CEA antibody moiety. However, among the four patients who had on-treatment $^{89}$Zr-PET scans in cycle 4, only one had detectable anti-drug antibodies at the time of imaging. This leaves the mechanism of the observed alterations in biodistribution in the other three patients unexplained. Regarding tumor accumulation, also occupation of CEA binding sites with unlabeled CEA-IL2v may explain the decreased accumulation although the short terminal half-life and relatively low dose of CEA-IL2v may argue against this[23].

**Conclusion**

In conclusion, this immuno-PET study confirms CEA-mediated tumor accumulation of CEA-IL2v with consistent accumulation at doses higher than 20 mg. In addition, CEA-IL2v distributes mainly to spleen and liver, generally independent of dose and CEA status. Repeated imaging during treatment allowed detection of altered biodistribution and tumor accumulation over time which is used to support selection of the optimal dose and regimen. Immuno-PET studies during different stages of treatment can thus improve the understanding of drug disposition, in particular target engagement, over time. CEA-IL2v is currently being developed in combination with the anti-PD-L1 antibody atezolizumab in CEA-expressing solid tumors (NCT02350673).

**ACKNOWLEDGEMENTS**

We thank all patients and their families for their participation; Roche for sponsoring; Martina Feilke, Cecile Guizani and Ernesto Guarin for their contributions to this study; Cenetron Central Laboratories for sample analysis; the departments of Pathology, Radiology and Nuclear Medicine and Medical Oncology of VUmc and the departments of Pathology, Pharmacology and Nuclear Medicine of The Netherlands Cancer Institute for support and conduct of this clinical trial.

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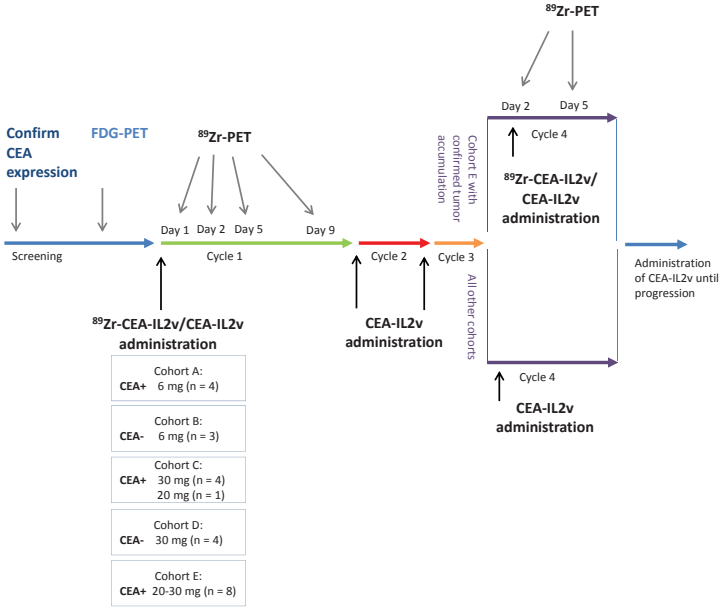
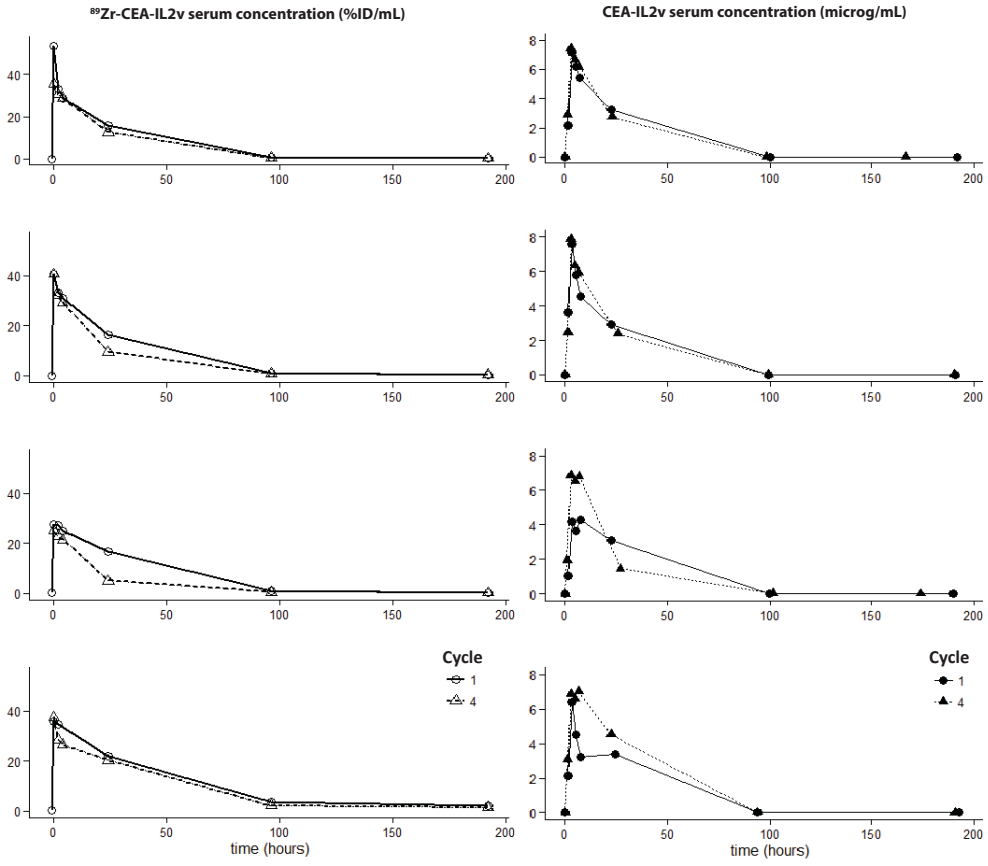
## SUPPLEMENTAL MATERIAL




**Supplemental Material – Figure 1.** Overview of study set-up and main study assessments.

**Supplemental Material - Table 1.** Patient characteristics. *ECOG PS, Eastern Cooperative Oncology Group Performance Status; CRC, colorectal carcinoma; NSCLC, non-small cell lung carcinoma; RCC, renal cell carcinoma.*

| | Patients (n=24) |
|---|---|
| **Age (years) [range]** | 65 [42-78] |
| **Gender (%)** | |
| Male | 58% |
| Female | 42% |
| **ECOG PS (%)** | |
| **0** | 43% |
| **1** | 57% |
| **Tumor type (*n*) (%)** | |
| ***CEA+*** | 17 |
| CRC | 11 (46%) |
| NSCLC | 4 (17%) |
| Salivary gland | 1 (4.2%) |
| Gastric | 1 (4.2%) |
| ***CEA-*** | 7 |
| RCC | 3 (13%) |
| Melanoma | 2 (8.3%) |
| Ovarian | 1 (4.2%) |
| Pancreatic cancer | 1 (4.2%) |




**Supplemental Material - Figure 2.** Individual whole blood concentration– time curves of $^{89}$Zr –CEA-IL2v in % injected dose/mL (%ID/mL; open symbols) and of unlabeled CEA-IL2v (µg/mL) (filled symbols) in cycle 1 (solid line) vs. cycle 4 (dashed line) for four patients treated with 20 in cycle 1 and 30 mg in cycle 4. The patient represented in the third row had detectable anti-drug antibodies at cycle 4.

**Supplemental Material - Table 2.** Biodistribution per dose cohort and by CEA status on cycle 1 day 5.

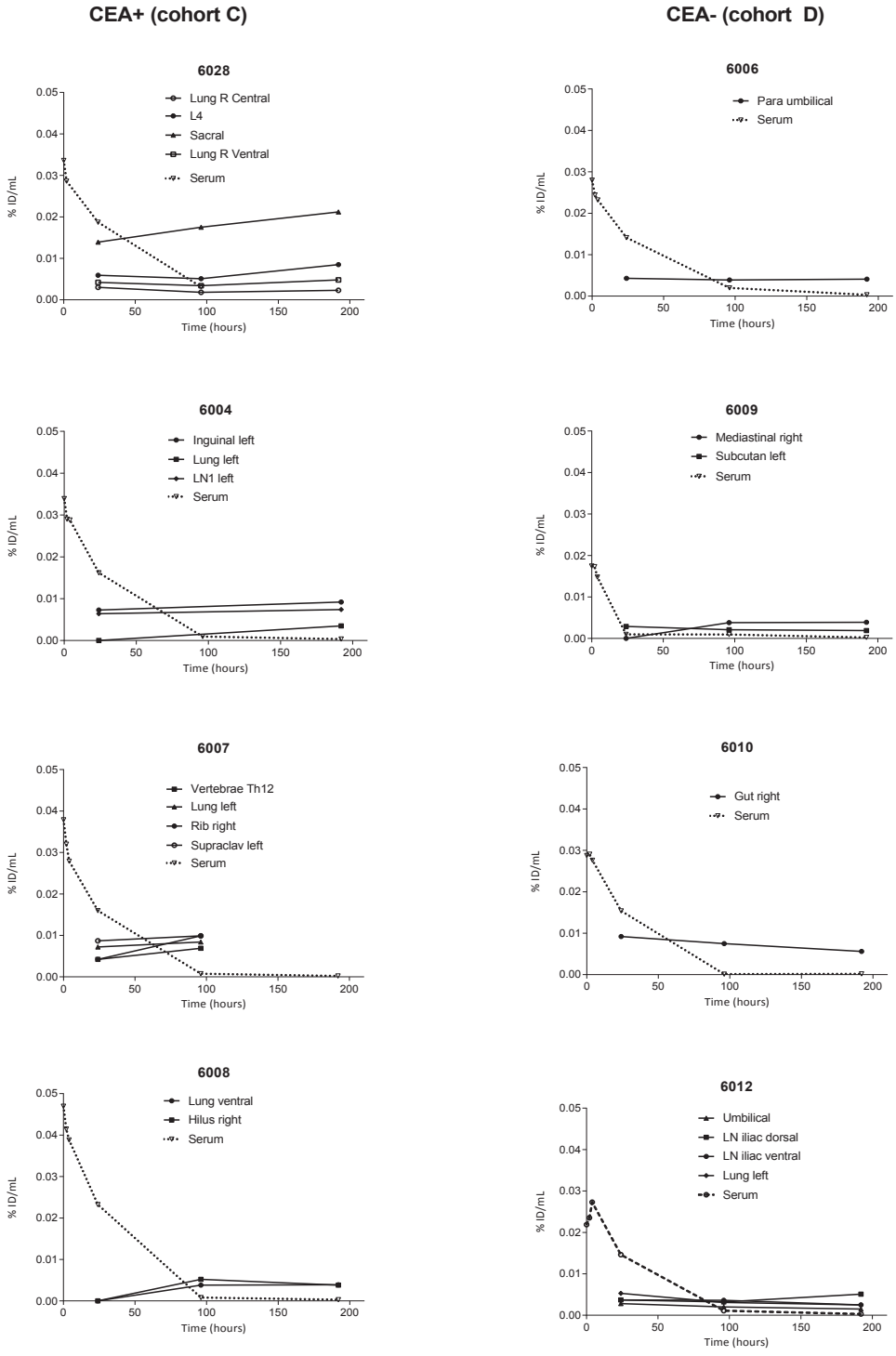
| | 6 mg | | 20 mg^ | 30 mg | | Total | | Overall |
|---|---|---|---|---|---|---|---|---|
| **Organ** (%ID/mL$_{mean}$ ±SD * $10^{-2}$) | **CEA+** (n=4) | **CEA-** (n=3) | **CEA+** (n=9) | **CEA+** (n=4) | **CEA-** (n=4) | **CEA+** (n=17) | **CEA-** (n=7) | |
| **Spleen** | 1.4 ± 0.23 | 1.6 ± 0.51 | 1.2 ± 0.30 | 0.78* ± 0.54 | 0.94* ± 0.25 | 1.2 ±0.40 | 1.2 ± 0.49 | 1.2 ± 0.42 |
| **Liver** | 1.1 ± 0.32 | 0.94 ± 0.22 | 1.2 ± 0.27 | 0.90 ± 0.65 | 1.1 ± 0.25 | 1.1 ± 0.39 | 1.01 ± 0.23 | 1.1 ± 0.35 |
| **Vertebrae** | 0.41 ± 0.11 | 0.46 ± 0.044 | 0.49 ± 0.15 | 0.40 ± 0.28 | 0.42 ± 0.086 | 0.45 ± 0.17 | 0.44 ± 0.068 | 0.44 ± 0.15 |
| **Kidneys** | 0.36 ± 0.058 | 0.39 ± 0.055 | 0.36 ± 0.070 | 0.26 ± 0.18 | 0.30 ± 0.015 | 0.34 ± 0.10 | 0.34 ± 0.059 | 0.34 ± 0.092 |
| **Lung** | 0.10 ± 0.010 | 0.10 ± 0.032 | 0.10 ± 0.030 | 0.072 ± 0.049 | 0.11 ± 0.024 | 0.10 ± 0.033 | 0.11 ± 0.026 | 0.10 ± 0.031 |

^ Including the 20 mg (n=1) and 20-30 mg cohort (n=8)

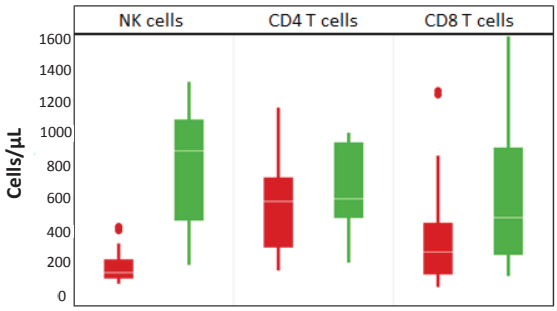
* Significant difference ($p$<0.05) assessed by One Way Analysis of Variance (ANOVA) comparing %ID/mL$_{mean}$ across 6 mg vs. 20 mg vs. 30 mg per organ and for CEA + vs. CEA- per organ

**Supplemental Material - Table 3.** Tumor accumulation; assessment of $^{89}$Zr-uptake in $^{18}$F-FDG+ lesions per patient. Accumulation was visually confirmed if $^{89}$Zr-positive lesions were visual at the cycle 1 day 5 plus one additional scan.
* Primary evaluation at cycle 1 day 2 because day 5 scan was not performed.

| Patient | No. of FDG positive lesions | No. of lesions $^{89}$Zr positive day 5 | Accumulation visually confirmed (Yes/No) | Consistence | CEA status (CEA+/CEA-) | Dose cycle 1 (mg) | Cohort |
|---|---|---|---|---|---|---|---|
| 1 | 7 | 1 | Yes | Negative on day 2 | CEA+ | 6 | A |
| 2 | 5 | 1 | Yes | Positive on day 2 | CEA+ | 6 | A |
| 3 | 5 | 0 | No | Consistent lack of accumulation | CEA+ | 6 | A |
| 4 | 11 | 7 | Yes | Consistent | CEA+ | 6 | A |
| 5 | 9 | 2 | No | Both negative on day 1 and 2 | CEA- | 6 | B |
| 6 | 6 | 0 | No | Consistent lack of accumulation | CEA- | 6 | B |
| 7 | 7 | 0 | No | Consistent lack of accumulation | CEA- | 6 | B |
| 8 | 7 | 5* | Yes | Positive on day 2 and 9, day 5 scan not performed | CEA+ | 30 | C |
| 9 | 4 | 4 | Yes | Consistent | CEA+ | 30 | C |
| 10 | 8 | 2 | Yes | Consistent | CEA+ | 20 | C |
| 11 | 10 | 6 | Yes | Consistent | CEA+ | 30 | C |
| 12 | 8 | 4 | Yes | Positive on day 5 and 9 | CEA+ | 30 | C |
| 13 | 7 | 2 | Yes | One lesion negative on day 2 (lymph node) | CEA- | 30 | D |
| 14 | 2 | 1 | Yes | Consistent | CEA- | 30 | D |
| 15 | 8 | 1 | Yes | Consistent | CEA- | 30 | D |
| 16 | 6 | 4 | Yes | Consistent | CEA- | 30 | D |
| 17 | 4 | 0 | No | Consistent lack of accumulation | CEA+ | 20 | E |
| 18 | 33 | 27 | Yes | Consistent | CEA+ | 20 | E |
| 19 | 7 | 7 | Yes | Consistent | CEA+ | 20 | E |
| 20 | 2 | Not evaluable | Not evaluable | | CEA+ | 20 | E |
| 21 | | | | | CEA+ | 20 | E |
| Cycle 1<br>Cycle 4 | 7<br>7 | 6<br>4 | Yes<br>Yes | Consistent<br>Consistent | | | |
| 22 | | | | | CEA+ | 20 | E |
| Cycle 1<br>Cycle 4 | 3<br>3 | 1<br>1 | Yes<br>Yes | Consistent<br>Consistent | | | |
| 23 | | | | | CEA+ | 20 | E |
| Cycle 1<br>Cycle 4 | 8<br>8 | 7<br>3 | Yes<br>Yes | Consistent<br>Consistent | | | |
| 24 | | | | | CEA+ | 20 | E |
| Cycle 1<br>Cycle 4 | 4<br>4 | 1<br>1 | Yes<br>Yes | Consistent<br>Consistent | | | |




**Supplemental Material - Figure 3.** Tumor uptake over time of CEA+ and CEA- patients treated with 30 mg (—— %ID/mL$_{peak}$) and serum pools (---- %ID/mL).




**Supplemental Material - Figure 4.** Lymphocyte counts (NK cells ($CD^{3+}CD^{56+}/CD^{16+}$), CD4 T cells ($CD^{3+}/CD^{4+}$) and CD8 T cells ($CD^{3+}/CD^{8+}$)) in cycle 1 (red) and cycle 4 (green) across all tested doses.

## Chapter 3.2

**Preliminary efficacy and safety of pembrolizumab in nine different solid tumor types**
An analysis of unmonitored data of patients treated in the Netherlands Cancer Institute

*Interim analysis*

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Marja Mergui
Mariska Bloedjes
Jos H. Beijnen
Jan H.M. Schellens

**ABSTRACT**

**Background** The inhibitory receptor programmed cell death 1 (PD-1) and its ligands, PD-L1 and PD-L2, are upregulated in many tumors and tumor-infiltrating immune cells, leading to suppression of antitumor immune responses. Pembrolizumab is a potent humanized monoclonal antibody against PD-1 that is designed to block the interaction between PD-1 and PD-L1/PD-L2. Antitumor effects of pembrolizumab have been established in advanced melanoma and non-small cell lung cancer, but the efficacy in many other tumor types is unknown. In the KEYNOTE-028 phase Ib trial, the efficacy and safety of pembrolizumab in 20 advanced solid tumor types was studied. In this paper, results from patients included in nine different cohorts in the Netherlands Cancer Institute are reported.

**Methods** For each of the 20 tumor types, 22 patients for whom standard therapy failed or was not feasible received 10 mg/kg pembrolizumab intravenously every two weeks until disease progression or unacceptable toxicity. Patients were recruited in in the Netherlands Cancer Institute and centers in the United States, Canada, France, Italy, Japan, Korea, Spain, Taiwan and the United Kingdom. Only patients with PD-L1 expression in ≥1% of cells in tumor nests or PD-L1–positive bands in stroma as determined by a prototype immunohistochemistry assay were included. Primary endpoints included safety, tolerability, and efficacy per RECIST v1.1.

**Results** In the Netherlands Cancer Institute, seventeen patients (pts) were enrolled in the cohorts anal canal, pancreatic, breast, vulvar, ovarian, small-cell lung and nasopharyngeal cancer, mesothelioma and carcinoid tumors. Median age was 64 years; 29% were male; 47% had a World Health Organization Performance Status of 1; all had received 1-5 lines of prior treatment lines for advanced disease. As of the data cut-off date of September 28th 2016, all pts had discontinued pembrolizumab due to disease progression. Treatment-related adverse events were all ≤ grade 3 and occurred in 14 pts, most commonly consisting of fatigue, fever, nausea and dry mouth. No pts died or discontinued pembrolizumab because of treatment-related adverse events. There were two partial responses in the nasopharyngeal and ovarian cohort. In the pancreatic, anal canal, mesothelioma, vulvar and carcinoid tumors cohorts, best response was stable disease. Median duration of stable disease was 6.5 months [range: 3.9-14]. Median PFS was 4.8 months [1.8-31+]; median OS was 5.9 months [1.4-12.5].

**Conclusion** Preliminary data show that pembrolizumab had promising antitumor activity in terms of partial responses and resulted in disease control in several tumor types, with a manageable safety profile.

## BACKGROUND

Pembrolizumab is a selective humanized monoclonal antibody that blocks the interaction between programmed death (PD)-1 receptor and its ligands PD-L1 and PD-L2. PD-L1 is expressed on a wide variety of tumor cells including but not limited to melanoma, non-small cell lung carcinoma (NSCLC), renal cell carcinoma, ovarian, pancreatic and colorectal cancer[1]. Expression of PD-L1 induces programmed death of PD-1 expressing cells which are mainly $CD^{4+}$ and $CD^{8+}$ T-cells, B-cells, natural killer (NK) cells and T-regulatory cells[2]. This enables tumor cells to avoid attack and elimination by these immune cells[2]. Higher expression of PD-L1 is correlated with better responses to anti-PD-1 therapy[1,3,4]. PD-L2 has a restricted expression pattern in lymphoid tissues and in chronic inflammations and is thought to control T-cell activation in lymphoid organs, making this interaction less relevant in cancer.

Monoclonal antibodies against PD-1 and PD-L1, including pembrolizumab, nivolumab, and atezolizumab, have demonstrated antitumor activity in a diverse set of tumor types, including melanoma, non-small cell lung cancer (NSCLC)-, bladder-, renal cell- and gastric cancer, and Hodgkin lymphoma[9-11]. Pembrolizumab is currently registered for use in metastatic melanoma, head and neck squamous cell carcinoma and NSCLC[5,6]. In these tumortypes, relevant and durable antitumor effects have been observed. In the pivotal trial in melanoma, pembrolizumab 2 mg/kg every three weeks induced an overall response rate (ORR) of 33% compared to 12% on ipilimumab (standard of care) and progression-free survival (PFS) was prolonged from 2.8 months on ipilumumab to 4.1 months on pembrolizumab[7]. In PD-L1 expressing NSCLC after first-line chemotherapy, the ORR was 18% in the pembrolizumab 2 mg/kg group compared to 9% on docetaxel, while overall survival was prolonged to 10.4 months compared to 8.5 months and the PFS of 4 months was the same in both groups[4].

In the KEYNOTE-028 phase Ib trial (NCT02054806), the efficacy and safety of pembrolizumab was studied in twenty different PD-L1 positive advanced or recurrent solid tumor types with unmet medical need (table 1). The KEYNOTE-028 trial finished enrollment in March 2016 and currently data of the 471 included patients are being analyzed. This interim analysis describes efficacy and toxicity data from patients that have been treated in the Netherlands Cancer Institute among nine different cohorts.

**Table 1.** Included PD-L1 positive tumor types

| Group | Tumor types |
|---|---|
| **Intestinal** | Colorectal, anal canal, pancreatic, esophageal, biliary, carcinoid, pancreas-derived neuroendocrine |
| **Female reproduction system** | Breast, ovarian, endometrial, cervical, vulvar |
| **Lung** | Small cell lung, mesothelioma |
| **Glands** | Thyroid, salivary gland |
| **Diverse** | Glioblastoma, nasopharyngeal, leiomyosarcoma, prostate |

## METHODS

### Study set-up

KEYNOTE-028 was a multicenter, open-label, phase 1b trial that included 20 cohorts of patients with PD-L1 positive advanced solid tumors. This study was executed at 12 investigational sites in the United States, Canada, France, Italy, Japan, Korea, Spain, Taiwan and the United Kingdom. The study protocol and all amendments were approved by the institutional review boards or ethics committees of all participating sites. All patients provided written informed consent. In this report, we describe results of the patients with mesothelioma, carcinoid tumors, anal canal carcinoma, pancreatic cancer, ovarian cancer, breast cancer, small-cell lung cancer, vulvar cancer, and nasopharyngeal carcinoma who were treated in the Netherlands Cancer Institute.

### Patient population

Patients were enrolled if they had histologically or cytologically confirmed, locally-advanced, or metastatic disease that was incurable and either failed on prior standard therapy, or for which no standard therapy existed or was not considered appropriate by the patient and treating physician. There was no limit to the number of prior treatment regimens. Additionally, tumors were required to be PD-L1 positive (≥1% of tumor or stroma cells or the presence of a distinctive interface pattern in both neoplastic cells and contiguous mononuclear inflammatory cells) as determined by a prototype immunohistochemistry (IHC) assay (QualTek Molecular Laboratories, Goleta, CA, USA) with the 22C3 antibody (Merck & Co, Kenilworth, NJ, USA) performed at a central laboratory from either an archived formalin-fixed, paraffin-embedded (FFPE) tumor sample or a newly obtained biopsy at screening.

Other eligibility requirements were age 18 years or older, measurable disease at baseline based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, World Health Organization (WHO) performance status of 0 or 1; and adequate organ function (hematological, renal, hepatic, and coagulation) as established with laboratory tests within ten days of the first pembrolizumab dose. Exclusion criteria included a diagnosis of immunodeficiency or systemic steroid therapy within seven days before the first pembrolizumab dose; receiving anticancer monoclonal antibodies within four weeks of the first pembrolizumab dose; active autoimmune disease; interstitial lung disease; active infection requiring systemic therapy; active brain metastases (metastases that were stable for ≥ four weeks before the first dose of pembrolizumab were permitted); chemotherapy, targeted small molecule therapy or radiation therapy within two weeks of first pembrolizumab dose; and previous therapy with antibodies against PD-1, PD-L1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), or any other immune checkpoint inhibitor.

### Treatment

Pembrolizumab was given intravenously at 10 mg/kg once every two weeks for two years or until confirmed disease progression, death, unacceptable toxicity, withdrawal of consent, or investigator decision to stop. This dose was selected based on the phase I trial KEYNOTE-001 in which 10 mg/kg was the highest tolerable and effective dose-level. Tumor imaging was done every eight weeks for the first six months and thereafter every 12 weeks by computed tomography (CT) or magnetic resonance imaging (MRI). Pembrolizumab was withheld if patients showed pre-specified drug-related toxic effects or severe or life-threatening adverse events (AEs), and was discontinued if AEs did not resolve to grades 0–1 within 12 weeks after the last infusion. Patients with AEs remaining at grade 2 after 12 weeks could continue pembrolizumab only if the AEs were asymptomatic and controlled.

### Outcomes

Baseline characteristics were extracted from the medical dossier including age, gender, WHO performance status, prior lines of treatment received and tumor type. The primary endpoint was overall response rate (ORR) by investigator assessment, defined as the proportion of patients having a confirmed complete response or partial response per RECIST v1.1 at any time during the study. Secondary objectives included safety and tolerability. For safety, AEs were monitored throughout the study and for 30 days after the end of treatment; serious adverse events (SAEs) were recorded for 90 days after the final dose of pembrolizumab. Immune-mediated AEs were defined as events of clinical interest with potentially drug-related immunological causes that were consistent with an immune phenomenon. All AEs were graded in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. For efficacy, outcomes were progression-free survival (PFS) reported as time from enrollment to the first documented disease progression according to RECIST v1.1 or death due to any cause, whichever occurred first; overall survival (OS); time to response (TTR) defined as time from start to first documented partial or complete response; and duration of response (DOR) defined as time from first RECIST v1.1-based response to disease progression in patients who achieved a partial response or better.

**Statistical analysis**

Efficacy was assessed in all patients who received at least one dose of pembrolizumab and had measurable disease at baseline according to RECIST v1.1. The safety analysis population included all patients who received at least one dose of pembrolizumab. The Kaplan-Meier method was used to estimate PFS, OS, and DOR. Patients were censored in case no event occurred at data cut-off or no evaluation was performed, or if they were lost to follow up. The data cutoff date for this report was September 28th 2016. All analyses were performed in R[8].

## RESULTS

Between February 2014 and March 2016, 17 patients were enrolled in the Netherlands Cancer Institute in the cohorts mesothelioma (n=4), carcinoid tumors (n=3), anal canal (n=2), pancreatic cancer (n=2), ovarian cancer (n=2), breast cancer (n=1), small-cell lung cancer (SCLC) (n=1), vulvar cancer (n=1), and nasopharyngeal carcinoma (n=1). Median age was 64 years [32-73], 29% were male, 47% had WHO Performance Status 1 and before study start patients had received median 1 [0-5] treatment line (table 2). All patients had discontinued pembrolizumab at the data cut-off.

**Table 2.** Patient characteristics. Continuous values reported as median [range].
*WHO PS, World Health Organization Performance Status; SCLC, small-cell lung cancer.*

| Demographic characteristic | Total (n=17) |
|---|---|
| **Age (years)** | 64 [32-73] |
| **Gender (%)** | |
| Male | 29 |
| Female | 71 |
| **WHO PS (%)** | |
| 0 | 53 |
| 1 | 47 |
| **Tumor type (n)** | |
| Mesothelioma | 4 |
| Carcinoid tumor | 3 |
| Anal canal | 2 |
| Pancreatic | 2 |
| Ovarian | 2 |
| Breast | 1 |
| Nasopharyngeal | 1 |
| SCLC | 1 |
| Vulvar | 1 |
| **Prior lines of therapy for advanced disease** | 1 [0-5] |

**Efficacy**

Antitumor activity of pembrolizumab (stable disease [SD] or partial response [PR]) was observed in nine out of 17 patients in six out of nine tumor types. PR was observed in two patients (figure 1) with a time to response (TTR) of 1.8 months in both patients (figure 2). Reductions in tumor volume of 74% were seen in the patient with nasopharyngeal carcinoma and in one out of two ovarian cancer patients. These patients were both pretreated; the nasopharyngeal carcinoma with one line of chemotherapy (cisplatin) and the ovarian cancer patient with five lines of hormonal and chemotherapies (tamoxifen, carboplatin, doxorubicin, cyclophosphamide, gemcitabin). Duration of response (DOR) was 6.2 months in the nasopharyngeal patient and >24 months in the patient with ovarian cancer, who completed the 24 months treatment-period and was taken off-study with an ongoing response. Among the four mesothelioma patients, the best response was stable disease which lasted median 4.4 months [1.8-26.8] (based on the time to progression of three patients).

SD was also observed in two out of three patients in the carcinoid cancer cohort and in the single patient with vulvar cancer and one with anal canal cancer. Overall, the median duration of SD was 6.5 months [3.9-14.0]. No responses were observed in the breast cancer and SCLC cohorts. In total, time on treatment was median 3.9 months [1.2-27] during which patients received median 8 [2-51] cycles. PFS was median 4.8 months [1.4->31 months] and overall survival median 5.9 months [1.4-12.5] (figure 3). The patients with a PR had a longer median PFS than the patients with SD (median 19.5 months vs. 5.8 months respectively) although statistical significance was not reached due to the small sample size.

Two patients (one pancreatic, one SCLC) were not evaluable because of discontinuation before the first tumor evaluation. Patients discontinued treatment due to radiologically confirmed or clinical progressive disease (PD) (n=15) or completion of the two year treatment period (n=2). No patients discontinued or died due to treatment-related toxicities.

**Safety**

Toxicities at least possibly related to treatment were observed in 14 out of 17 patients as presented in table 3.

The most common treatment-related AEs were fatigue (n=9), nausea (n=4), dry mouth (n=3) and fever (n=3). There were three cases of grade 3 AEs including increased bilirubin (n=1), proteinuria (n=1) and uveitis (n=1). No events of grade 4 of higher were reported. No late-onset treatment-related toxicities were reported after treatment-discontinuation.

Events of special interest that are likely to be immune-related occurred in six patients; uveitis grade 1 and 3, pruritus grade 1 and 2, and hypothyroidism grade 2 occurred in two patients each and aspartate aminotransferase (ASAT)/ alanine aminotransferase (ALAT) elevation grade 1 was observed in one patient. Grade 1 uveitis occurred after 12 cycles and recovered without supporting treatment. Grade 3 uveitis occurred after the first administration of pembrolizumab. Local corticosteroids were given and pembrolizumab was interrupted for two weeks. Symptoms recurred intermittently but could be managed with local corticosteroids without affecting treatment continuation. Pruritus was reported after two cycles in one patient and after 18 cycles in the other patient. In both patients symptoms were managed with local application of soothing cream and treatment was continued as planned. Hypothyroidism occurred in two patients, after respectively two and four cycles, and levothyroxine was started to replace thyroid function. The grade 2 ASAT/ALAT elevation occurred after two cycles while the patient concomitantly used maximum daily doses of acetaminophen. ASAT/ALAT normalized after acetaminophen treatment was stopped. The observed toxicities were comparable across the different cohorts and in line with the results of other clinical trials.

## DISCUSSION

The efficacy and safety data of the 17 patients described in this report should be interpreted in context with the preliminary overall study results. Unpublished results indicate that efficacy is observed in seven out of twenty tumor types that were included in the study. Overall response rates in these cohorts were; 12% in ovarian cancer, 12% in breast cancer, 29% in SCLC, 28% in mesothelioma, 26% in nasopharyngeal carcinoma, 20% in anal canal carcinoma and 0% in pancreatic cancer. In our data, promising antitumor activity in terms of partial responses were seen in nasopharyngeal and ovarian cancer which is in line with the overall data. For mesothelioma, anal canal carcinoma, breast cancer, and SCLC the overall response rates are not reflected in our data. Among three mesothelioma patients, the best response was prolonged disease stabilization for 4 to 27 months. From the two patients with anal canal carcinoma, only stable disease was observed in one patient. In the breast cancer and in the SCLC patient, disease progression was observed at the first evaluation after two treatment cycles. The discrepancy between the overall study data and this subset analysis could be explained by the small number of patients per cohort that were included in this manuscript, leading to an underpowered analysis. Additionally, response may be affected by inter-individual differences in disease status, although no apparent correlation could be found in our data.

Similar to the results of other trials in which checkpoint inhibitors are studied, only a subset of patients with PD-L1 positive cancer showed clinical benefit of PD-1 pathway blockade in our study. An increasing load of evidence suggests that the use of tumor PD-L1 expression as a biomarker may be insufficiently predictive for response. Whereas some studies confirm that a high PD-L1 expression is associated with higher response rates[3,4,9,10] other studies suggest that there is no true association[11–13]. The use of PD-L1 as a biomarker is hindered by the variability in assay results, the dynamic nature of PD-L1 expression and the variability in cut-off values for PD-L1 positivity. These factors underlie inconsistent data on the predictive value of PD-L1 expression. Additionally, numerous other characteristics of the tumor and the immune response may be relevant for prediction of successful antitumor activity with checkpoint inhibitors including PD-1 blockade[14,15]. Regarding tumor characteristics, mutational load has been reported to correlate with clinical benefit with immune checkpoint blockade in melanoma, NSCLC, and colorectal cancer and possibly in other types of cancer too[16–19].

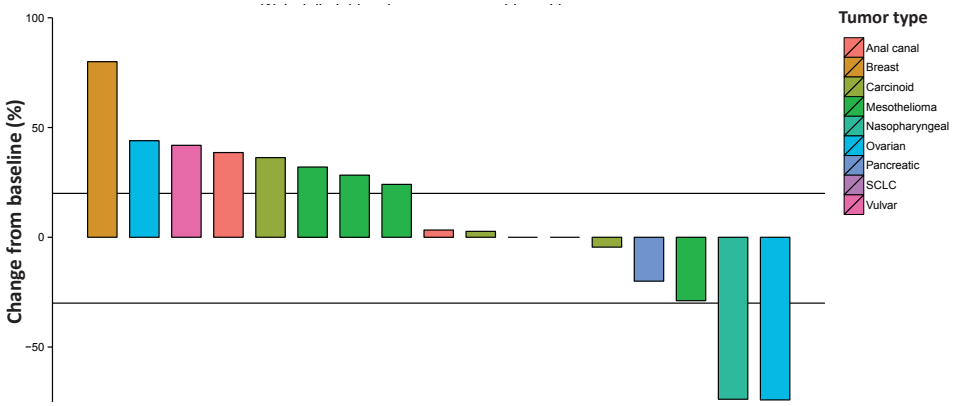



**Figure 1**. Best response for each patient presented as maximum change from baseline in percentage.

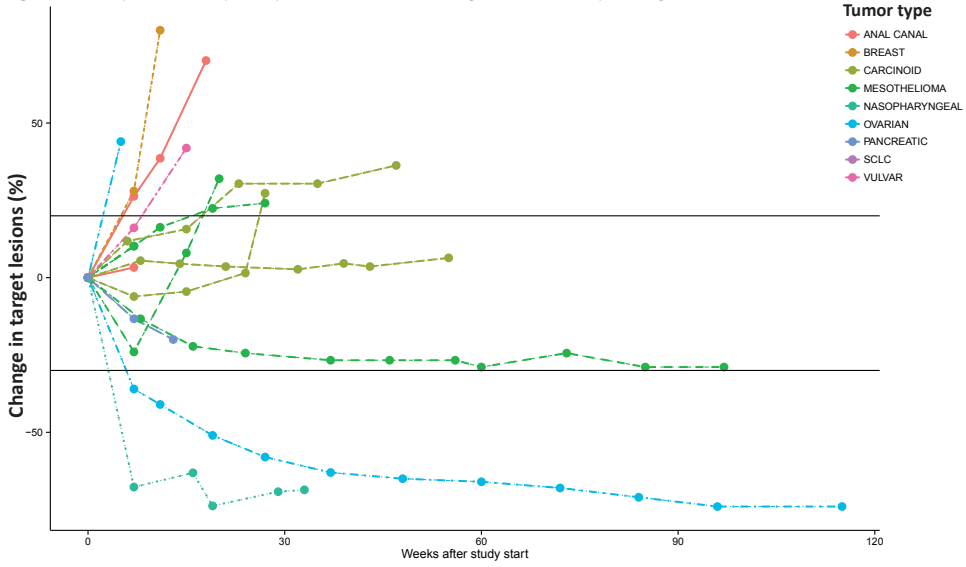
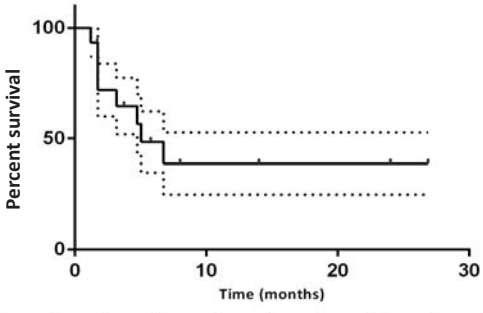



**Figure 2.** Changes in sum of target lesion diameters over time according to RECIST 1.1. *SCLC, small-cell lung cancer.*




| Month | 0.0 | 1.2 | 1.7 | 1.8 | 3.2 | 3.8 | 4.8 | 5.0 | 5.8 | 6.8 | 8.0 | 14.0 | 24.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number at risk | 15 | 15 | 14 | 13 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 |

**Figure 3.** Survival percentages (-----) with standard error (·····) of the included population.

**Table 3.** Toxicities at least possibly related to pembrolizumab occurring during and up to 28 days after treatment.

| Toxicity | Grade 1-2 ( n) | Grade 3 (n) | Total (n (%)) |
|---|---|---|---|
| Allergic reaction | 1 | | 1 (6) |
| Alkaline phosphatase increased | 1 | | 1 (6) |
| Arthralgia | 1 | | 1 (6) |
| ASAT/ALAT increased** | 1 | | 1 (6) |
| Balance disorder | 1 | | 1 (6) |
| Bilirubin increased | 1 | 1 | 2 (12) |
| Dry eyes | 1 | | 1 (6) |
| Dry mouth | 3 | | 3 (18) |
| Dysgeusia | 2 | | 2 (12) |
| Dyspnea | 2 | | 2 (12) |
| Fatigue | 9 | | 9 (53) |
| Fever | 3 | | 3 (18) |
| Flu-like symptoms | 1 | | 1 (6) |
| Flushing | 1 | | 1 (6) |
| Hypocalciemia | 1 | | 1 (6) |
| Hypothyreoidism** | 2 | | 2 (12) |
| Infusion-related reaction | 2 | | 2 (12) |
| Light flashes | 1 | | 1 (6) |
| Nail loss | 1 | | 1 (6) |
| Nausea | 4 | | 4 (24) |
| Obstipation | 1 | | 1 (6) |
| Oral pain | 1 | | 1 (6) |
| Pain in extremity | 1 | | 1 (6) |
| Proteinuria | 1 | 1 | 2 (12) |
| Pruritus** | 2 | | 2 (12) |
| Stomach/abdominal pain | 2 | | 2 (12) |
| Sweating/night sweats | 2 | | 2 (12) |
| Thrombo-embolic event | 2 | | 2 (12) |
| Trombocytopenia | 1 | | 1 (6) |
| Uveitis** | 1 | 1 | 2 (12) |
| Weight loss | 1 | | 1 (6) |

*** Immune-related toxicities of special interest*

A higher mutational load may increase the chance of T-cell antigen recognition. Secondly, tumor infiltration with $CD^{8+}$ and $CD^{3+}$ T cells was associated with response to PD-1 inhibition, in addition to PD-1 and PD-L1 expression on tumor epithelial cells[14]. Also T-regulatory cells may play an important role as they can suppress immune responses[14,15].

Systemically, counts of lymphocytes, neutrophils, eosinophils, $CD^{8+}$ T-cell clones and expansion rates have been reported to correlate with the response to immune checkpoint inhibitors[14]. Higher counts of systemical immune cells or activation markers may be indicators of an effective immune response in general and may be related to intratumoral immune infiltration too. Taken together, the use of tumor-PD-L1 expression as a biomarker may be too limited whereas a signature profile including PD-L1 expression, mutational load and systemical or intratumoral lymphocytes may provide a better prediction for response. The data collection to facilitate the development of this signature is ongoing. Unfortunately, from the patients included in this analysis, biomarker

data were not available at the time of data cut-off. The identification of the most reliable set of predictive biomarkers will help optimizing patient benefit and decreasing treatment costs.

In conclusion, the preliminary data from this KEYNOTE-028 trial show that pembrolizumab had promising antitumor activity (PR) and resulted in disease control (SD) in several tumor types, with a manageable safety profile. The results of the other tumor types included in this trial and not described in this report are expected soon because patient enrollment has been finished. Pembrolizumab is being developed further for the tumor types in which activity was confirmed. In the ongoing KEYNOTE-158 trial, biomarkers and signs of efficacy are studied in anal canal carcinoma, cervical cancer, small-cell lung carcinoma, vulvar carcinoma and mesothelioma. The KEYNOTE-122 trial evaluates pembrolizumab in platinum-pretreated nasopharyngeal cancer. In addition, different combination strategies are evaluated in pancreatic and breast cancer[20]. Also for tumor types in which efficacy was not observed, combination strategies may be feasible. Bendell *et al.* reported that resistance of microsatellite stable colorectal cancer to anti-PD-L1 treatment can be overcome by combining anti-PD-L1 with a MEK inhibitor to induce T-cell infiltration[21]. Combining pembrolizumab with agents that induce antigen presentation and hereby increase T-cell recognition may improve efficacy for tumor types that are poorly responsive to pembrolizumab as single agent. This combination may consist of pembrolizumab with a targeted agent as described by Bendell *et al.*[21], but may also be concomitant radiotherapy or chemotherapy which both have proven to increase mutational load and antigen presentation[22,23].

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## Chapter 3.3

**Clinical safety and efficacy of pembrolizumab in patients with malignant pleural mesothelioma: preliminary results from the phase 1b, non-randomised, open-label KEYNOTE-028 trial**

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**ABSTRACT**

**Background**
Malignant pleural mesothelioma (MPM) is a highly aggressive cancer with poor prognosis and limited treatment options following progression on platinum-containing chemotherapy. The multi-cohort phase 1b KEYNOTE-028 trial evaluated the safety and efficacy of pembrolizumab (an anti–programmed death 1 receptor [PD-1] antibody) in advanced solid tumours expressing PD-1 ligand 1 (PD-L1).

**Methods**
Previously treated patients with PD-L1–positive MPM were enrolled from 13 centres in six countries. Patients received pembrolizumab (10 mg/kg every two weeks) for up to two years or until confirmed progression or unacceptable toxicity. Key eligibility criteria included measurable disease, failure of standard therapy, and Eastern Cooperative Oncology Group preformance status of 0 or 1. PD-L1 positivity was defined as expression in ≥1% of tumour cells by immunohistochemistry. Response was assessed based on investigator review using the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1). Primary endpoints were safety and tolerability, analysed in the all-patients-as-treated population, and objective response, analysed for the full-analysis set. This trial is registered with ClinicalTrials.gov, number NCT02054806, is ongoing but not recruiting participants.

**Results**
As of June 20, 2016, 25 patients received pembrolizumab. Sixteen (64·0%) patients reported a treatment-related adverse event; the most common were fatigue (six [24%]), nausea (six [24%]), and arthralgia (five [20%]). Five (20%) patients reported grade 3 treatment-related adverse events. Three (12%) patients required dose interruption because of immune-related adverse events: one (4%) of 25 each had grade 3 rhabdomyolysis and grade 2 hypothyroidism; grade 3 iridocyclitis, grade 1 erythema multiforme, and grade 3 erythema; and grade 2 infusion-related reaction. No treatment-related deaths or discontinuations occurred. Five (20%) patients had a partial response, for an objective response of 20% (95% CI 6·8–40·7%), and 13 (52%) had stable disease. Responses were durable (median response duration 12·0 months [95% CI 3·7 months to not reached]); two patients remained on treatment at data cutoff.

**Conclusion**
Pembrolizumab appears to be well tolerated and may confer anti-tumour activity in patients with PD-L1–positive MPM. Response durability and efficacy in this patient population warrants further investigation.

## INTRODUCTION

Malignant pleural mesothelioma (MPM) is a rare and aggressive cancer that develops in the mesothelial lining of the pleura. Pleural mesothelioma is the most common of the malignant mesotheliomas, accounting for ~90% of cases.[1,2] Histological subtypes of MPM include epithelioid (~60%) and non-epithelioid (~40%)[3] variants; the latter includes subtypes of spindle, sarcomatoid, desmoplastic mesothelioma, fibrous mesothelioma, biphasic, and not otherwise specified.[2] Incidence varies widely both within and between countries: 29 per 1,000,000 people per year in Australia and the United Kingdom, ten per 1,000,000 people per year in the United States of America, and eight per 1,000,000 in Japan.[1]

MPM is caused primarily by inhalation of asbestos (~80% of cases).[1] It has a poor prognosis, in part because it is often diagnosed at a late stage.[2] Median survival for untreated MPM is usually less than 1 year.[2,4] Treatments include palliative surgical resection, radiation therapy, chemotherapy, or pleurodesis.[4] Disease is resectable in only 10–15% of patients.[2] Currently approved first-line therapy is chemotherapy with pemetrexed plus cisplatin,[5–7] which was associated with median overall survival (OS) of approximately 12 months.[7] There is currently no approved second line therapy. Therapeutic options following inadequate response of first-line therapy are pemetrexed (only if pemetrexed-naive in first line), platinum chemotherapy rechallenge (if patients had response in first line), vinorelbine or gemcitabine monotherapy, or participation in a clinical trial.[5,8] Median OS for second-line therapy ranges from 5·7 months to 10·9 months.[9–14]

Engagement of programmed cell death receptor 1 (PD-1), which is expressed on activated T-cells, with its ligands PD-L1 and PD-L2, limits T-cell effector functions.[15] Tumours can bypass anti-tumour responses by over-expressing PD-L1.[15] Pembrolizumab is a high-affinity, humanized monoclonal antibody against PD-1 designed to block the interaction between PD-1 and both PD-L1 and PD-L2.[16] It has shown robust and durable anti-tumour activity and a favourable safety profile in multiple tumour types.[16] Pembrolizumab is approved in more than 60 countries for one or more advanced malignancies, including advanced non small-cell lung cancer (NSCLC) with PD-L1 tumour proportion score ≥ 50%, no epidermal growth factor receptor or anaplastic lymphoma kinase genomic aberrations, and no prior systemic therapy; and advanced NSCLC with PD-L1 tumour proportion score ≥ 1% that has progressed on or after platinum-containing chemotherapy.[18,19]

PD-L1 is expressed in 20–40% of patients with MPM and appears to be more common in non-epithelioid tumours.[20,21] PD-L1–positive MPM appears to be associated with worse prognosis than is PD-L1–negative disease (median survival 4·8–5·0 months vs 14·5–16·3 months)[20,21] and is an independent risk factor for OS (relative risk 1·71 95%CI 1·03 - 2·79; $p$=0.04).[21] These findings raise the possibility that immune checkpoint inhibitor therapy could be an effective treatment option in this patient population.

In the phase 1b KEYNOTE-028 trial (ClinicalTrials.gov identifier: NCT02054806), the anti-tumour activity and safety of pembrolizumab were assessed in patients with PD-L1–positive advanced solid tumours. We report an interim analysis of these safety and activity data for the cohort with PD-L1 positive MPM.

## METHODS

### Study design and participants

KEYNOTE-028 is an ongoing phase 1b, multicentre, non-randomised, open-label, and multi-cohort trial of pembrolizumab in patients with PD-L1–positive advanced solid tumours, including MPM. Patients in the MPM cohort were enrolled from 13 centers in six countries. Patients were aged 18 years or older, had histologically confirmed locally advanced or metastatic MPM (diagnosis for each patient was based on histological and immunohistochemical examination of a pleural biopsy specimen) that is incurable, and had either failed standard therapy or were unable to receive standard therapy. Additional inclusion criteria were: measurable disease per Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST v1.1), Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, PD-L1–positive tumour, adequate organ function, no autoimmune or interstitial lung disease, and no active brain metastases. PD-L1 expression was assessed in archival or new tumour samples by a central laboratory (QualTek Molecular Laboratories, Goleta, CA, USA) with a prototype assay and the 22C3 antibody (Merck, Kenilworth, NJ, USA).[22,23] PD-L1 positivity was defined by membranous PD-L1 expression in at least 1% of tumour and associated inflammatory cells, or positive staining in stroma. Key exclusion criteria were having received previous treatment with PD-1 and PD-L1 checkpoint inhibitors, additional malignancy requiring treatment (except basal cell or squamous cell carcinomas of the skin or in situ cervical cancer), known active brain metastases (metastases that were stable for at least four weeks before the first dose of pembrolizumab were permitted) or carcinomatous meningitis, active autoimmune disease requiring systemic

steroids, interstitial lung disease, history of human immunodeficiency virus infection, and known active hepatitis B or hepatitis C virus infection. All patients provided written informed consent to participate before initiation of the study drug per investigational review board requirements. Enrolment progressed only after the first six patients had submitted to at least one post-baseline scan to assess tumour response, and two of those had a confirmed or unconfirmed response. A protocol-specified sequential monitoring procedure was subsequently used to evaluate for activity and futility simultaneously, based on the number of patients with a confirmed or unconfirmed response.

**Procedures**

Patients received 10 mg/kg pembrolizumab (Merck, Kenilworth, NJ, USA) intravenously every two weeks on day 1 of each cycle until documented disease progression, intolerable toxicity, physician decision, withdrawal of consent, or they reached the maximum of 24 months of pembrolizumab treatment. This dose was selected based on the highest tested dose in the open-label phase 1 KEYNOTE-001 trial of pembrolizumab in advanced malignancies, which showed no maximum tolerated dose to date, and no difference in efficacy or safety between doses of 10 mg/kg every 2 or 3 weeks and 2 mg/kg every 3 weeks.[18,24-27] Pembrolizumab was withheld in participants with intolerable effects, but could be resumed after resolution of that toxicity to grade 1 or baseline. If such resolution did not occur within 12 weeks after the last infusion, pembrolizumab was discontinued. Tumour imaging by computed tomography or magnetic resonance imaging (the same technique was used throughout the trial for individual patients) was performed at each site every eight weeks for the first six months, and then every 12 weeks thereafter. Adverse events (AEs) were monitored throughout the study period and graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0). Laboratory tests for haematology, chemistry and urinalysis were done for screening within ten days before the first dose of pembrolizumab, and then up to 72 hours before subsequent doses. Local institutional normal value was used as cutoff for each test.

**Study Outcomes**

The primary endpoints were safety and tolerability (as graded by CTCAE v4.0), and confirmed objective response (OR; per RECIST v1.1 based on investigator review). OR was defined as the percentage of evaluable patients with complete response (CR) and partial response (PR). Progression-free survival (PFS), OS, and duration of response (DOR) were secondary endpoints. PFS was defined as time from allocation to treatment to the first documented disease progression according to RECIST v1.1 or death due to any cause, whichever occured first. OS was defined as the time from allocation to treatment to death due to any cause. DOR was defined as time from the first evidence of response per RECIST v1.1 to disease progression in patients who achieved a PR or better. Data regarding the time between initial pathological diagnosis and first dose of pembrolizumab and PFS from first diagnosis could not be obtained because of the multicohort nature of this phase 1b study, which included 20 different malignancies.

**Statistical analysis**

The aim was to enrol approximately 22 patients, thus providing 80% power to demonstrate that the OR induced by pembrolizumab exceeded 10% at an overall one-sided 8% alpha level, assuming the true proportion for a given tumour type was 35%. Investigator-assessed OR was analysed using the truncated sequential probability ratio test, which provided the point estimate, repeated confidence intervals at 95%, and adjusted p values, with data from the full analysis set (all patients who received at least one dose of pembrolizumab and had measurable disease per RECIST v1.1 at baseline). PFS was analysed with Kaplan-Meier summary statistics with data from the full analysis set. DOR was analysed with Kaplan-Meier summary statistics with data from the full analysis set and all responders. OS was analysed with the Kaplan-Meier method with data from the full analysis set. Data were statistically analysed with SAS (version 9.3). The primary safety endpoint data, AEs (including serious AEs and AEs of immune-related cause) graded with CTCAE v4.0, were analysed in the all-patients-as-treated population (who had received at least one dose of pembrolizumab and attended at least one follow-up visit), and are summarised with descriptive statistics. The data cutoff for this interim analysis was June 20, 2016. This interim analysis date was prespecified and done to report favourable activity observed in the mesothelioma cohort rather than waiting for the final database lock for all cohorts involved in the study (estimated date: September 29, 2017).

**Role of the funding source**

Merck funded, administered, and sponsored the study, which was designed by the academic authors in conjunction with representatives of Merck. Data were collected by Merck and analysed in collaboration with the authors. EWA, AM, SS, and BP had access to the raw data. All of the authors vouch for the accuracy and completeness of the data. All authors made the decision to submit the manuscript for publication.

## RESULTS

### Patient baseline characteristics and disposition

Of the 83 patients with MPM who were screened, 38 (46%) had PD-L1–expressing tumours, and 25 (66%) of these patients were eligible for inclusion in the study (figure 1). Post-baseline scans done after the first six patients had been enrolled and received pembrolizumab showed confirmed or unconfirmed tumour responses in two patients; this finding, and the subsequent protocol-specified sequential monitoring procedure, led to the eventual enrolment and treatment of 25 patients enrolled between May 13, 2014, and August 20, 2014.

Table 1 summarises the baseline characteristics of the patients in this cohort. 18 (72%) patients had histologically confirmed epithelioid mesothelioma, two (8%) had biphasic histology, two (8%) had sarcomatoid histology, and three (12%) did not have histology specified. At the time of data cutoff (June 20, 2016), 21 (84%) had discontinued treatment; two (8%) remained on pembrolizumab, and two (8%) had completed the protocol-specified maximum 24 months of treatment. 14 (56%) patients died; nine (36%) due to disease progression, one (4%) suspected sepsis and suspected pulmonary embolism (same patient), and four (16%) for reasons unknown.

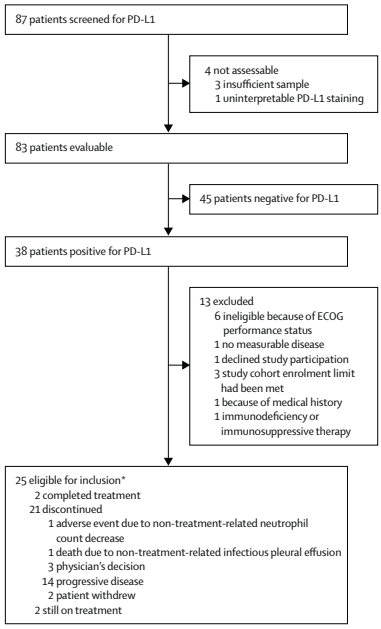



**Figure 1.** Programmed death receptor 1 ligand 1 (PD-L1) screening for the malignant pleural mesothelioma cohort. Reasons for discontinuation are to be confirmed at study completion.
*Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death receptor ligand 1; * Patients treated as of June 20, 2016.*

| Characteristic | Patients (n=25) |
|---|---|
| **Median age (years) (range)** | 65 (57-73) |
| **Sex** | |
| Male | 17 (68%) |
| Female | 8 (32%) |
| **Prior lines of therapy** | |
| 0 | 2 (8%) |
| 1 | 15 (60%) |
| ≥2 | 8 (32%) |
| **Histology** | |
| Epithelioid | 18 (72%) |
| Sarcomatoid | 2 (8%) |
| Biphasic | 2 (8%) |
| Not specified/reported[a] | 3 (12%) |
| **ECOG PS** | |
| 0 | 9 (36%) |
| 1 | 16 (64%) |
| **Race** | |
| White | 21 (84%) |
| Asian | 2 (8%) |
| Unknown | 2 (8%) |
| **Prior chemotherapy** | |
| Cisplatin/carboplatin | 22 (88%) |
| Pemetrexed | 21 (84%) |
| Gemcitabine | 4 (16%) |
| Vinorelbine | 1 (4%) |

**Table 1.** Demographic characteristics. *[a]Histological subtype of three patients was not provided in the pathology report; however, their diagnosis of malignant pleural mesothelioma was confirmed histologically and by immunohistochemical staining at screening, per protocol. ECOG PS, Eastern Cooperative Oncology Group Performance Status.*

All 25 patients received at least one dose of pembrolizumab. The duration of exposure is measured from the date of the first dose to the date of the last dose of treatment received at the time of data cutoff. The median duration of therapy was 5.1 months (interquartile range [IQR], 1·9–13·0). 12 (48%) patients had a duration of exposure that lasted 6 months or longer. Dose reductions were required by one (4%) of the 25 patients because of elevated gamma-glutamyltransferase and alanine aminotransferase. Their dose was reduced from 10 mg/kg every two weeks to 10 mg/kg every three weeks. Median follow-up duration was 18·7 months (IQR 9·4–24·2).

**Safety and tolerability**

Sixteen (64%) patients reported treatment-related AEs (table 2). The most frequently observed treatment-related AEs (any grade) in at least 10% of patients were fatigue, nausea, arthralgia, pruritus, decreased appetite and dry mouth (table 2). Five (20%) patients reported grade 3 treatment-related adverse events: thrombocytopenia, iridocyclitis, dyspnoea, alanine aminotransferase increase (one patient for each); and decrease in neutrophil count, decrease in appetite, and pyrexia (same patient). There were no drug-related deaths and no discontinuations due to treatment-related AEs.

Immune-related AEs were observed in three (12%) patients and included one each of the following: grade 2 infusion-related reaction; grade 2 hypothyroidism and grade 3 rhabdomyolysis (same patient); and grade 3 erythema, grade 1 erythema multiforme and grade 3 iridocyclitis (same patient). Each of these three patients required dose interruption because of these particular AEs. Treatment was reinitiated for all three patients until 24 months of treatment was completed, or until disease progression. No drug-related deaths or discontinuations resulted from the immune-related AEs.

**Anti-tumour activity**

The number of patients with a confirmed OR was 20% (95% CI 6·8–40·7), and the clinical benefit (i.e. CR + PR + stable disease [SD] for at least 6 months) was 40% (95% CI 21·1–61·3, table 3). Five (20%) patients achieved a PR, and 13 (52%) patients had SD; no patient had a CR. The median duration of SD was 5·6 months (95% CI 3·6–12·0). Disease progression as the best response assessment was observed in four (16%) patients. Median time to response in the five (20%) patients with a PR was 1·9 months (95% CI 1·7–3·8). Reduction in tumour size from baseline in target lesions was observed in 14 (56%) of 23 patients; two were not assessable as they discontinued pembrolizumab treatment at the day of their enrolment and they did not have any scans, and one patient had a response of stable disease but was recorded as not evaluable because this response was not confirmed (figure 2). Although two additional patients had reduction in tumour size more than 30%, meeting the criteria for partial response by RECIST, they did not have subsequent imaging that confirmed PR and were not included in the confirmed ORR reported above. Median DOR was 12·0 months (95% CI 3·7 to not reached). Three responses were ongoing at the time of data cutoff (figure 2), including two patients who had completed 24 months of protocol-specified therapy. Median PFS was 5·4 months (95% CI 3·4–7·5); 6-month PFS was 45·8% (25·6-64·0) and 12-month PFS was 20.8·8% (7·6-38·5; figure 3 and table 3). Median OS was 18·0 months (95% CI 9·4 to not reached); 6-month OS 83.5% (61·7-93·5) and 12-month OS was 62·6% (40·4-78·5; figure 3 and table 3).

**Table 3.** Confirmed pembrolizumab efficacy in the full analysis population per RECIST. Data are n(%), % (95% CI), unless otherwise stated. *Abbreviations: CI, confidence interval; CR, complete response; NR, not reached; OS, overall survival; PFS, progression-free survival; PR, partial response; RECIST v1.1, Response Evaluation Criteria in Solid Tumors version 1.1; SD, stable disease*
*[a]Two patients were listed as no assessment because they discontinued at the day of their enrollment and they did not have any scans.*

| | Full analysis set (n=25) |
|---|---|
| Objective response | 5 (20%; 95% CI 6·8–40·7) |
| Complete response | 0 |
| Partial response | 5 (20%) |
| Stable disease | 13 (52%) |
| Progressive disease | 4 (16%) |
| Not evaluable/no assessment[a] | 3 (12%) |
| Duration of follow-up (months) | 18·7 (10·4–24·0) |
| Time to response (months) | 1·9 (1·7-3·8) |
| Duration of response (months) | 12·0 (3·7–not reached) |
| Duration of stable disease (months) | 5·6 (3·6–12·0) |
| Clinical benefit rate (CR+PR+SD≥6 months) | 40% (21·1–61·3) |
| Progression-free survival | |
| Events | 21 (84·0) |
| Median (months) | 5·4 (3·4–7·5) |
| 6-months | 45·8 % (25·6-64·0) |
| 12-months | 20·8% (7·6-38·5) |
| Overall survival | |
| Deaths | 14 (56·0) |
| Median (months) | 18·0 (9·4–NR) |
| 6-months | 83·5 (61·7-93·5) |
| 12-months | 62·6 (40·4-78·5) |

**Table 2.** All treatment-related adverse events observed in patients. Data are n (%). n=25. No grade 4 or 5 treatment related adverse events occurred.

| Adverse event, n (%) | Grade 1 | Grade 2 | Grade 3 |
|---|---|---|---|
| Nausea | 5 (20%) | 1 (4%) | - |
| Fatigue | 3 (12%) | 3 (12%) | - |
| Arthralgia | 2 (8%) | 3 (12%) | - |
| Pruritus | 2 (8%) | 2 (8%) | - |
| Decreased appetite | 1 (4%) | 1 (4%) | 1 (4%) |
| Dry mouth | 3 (12%) | - | - |
| Asthenia | - | 2 (8%) | - |
| Constipation | 2 (8%) | - | - |
| Diarrhoea | 1 (4%) | 1 (4%) | - |
| Dry skin | 2 (8%) | - | - |
| Dyspnoea | - | 1 (4%) | 1 (4%) |
| Headache | 1 (4%) | 1 (4%) | - |
| Mucosal inflammation | 2 (8%) | - | - |
| Pyrexia | 1 (4%) | - | 1 (4%) |
| Rash maculopapular | 2 (8%) | - | - |
| Alanine aminotransferase increased | - | - | 1 (4%) |
| Aspartate aminotransferase increased | - | 1 (4%) | - |
| Balance disorder | 1 (4%) | - | - |
| Blood alkaline phosphatase increased | 1 (4%) | - | - |
| Burning sensation | 1 (4%) | - | - |
| Cancer pain | - | 1 (4%) | - |
| Chest pain | 1 (4%) | - | - |
| Cough | 1 (4%) | - | - |
| Dysgeusia | - | 1 (4%) | - |
| Gamma-glutamyltransferase increased | - | 1 (4%) | - |
| Haemoglobin decreased | 1 (4%) | - | - |
| Hypocalcaemia | 1 (4%) | - | - |
| Infusion-related reaction | - | 1 (4%) | - |
| Iridocyclitis | - | - | 1 (4%) |
| Irritability | 1 (4%) | - | - |
| Joint stiffness | 1 (4%) | - | - |
| Musculoskeletal stiffness | 1 (4%) | - | - |
| Myalgia | 1 (4%) | - | - |
| Neutrophil count decreased | - | - | 1 (4%) |
| Paraesthesia | 1 (4%) | - | - |
| Platelet count decreased | 1 (4%) | - | - |
| Pleuritic pain | - | 1 (4%) | - |
| Rash | 1 (4%) | - | - |
| Rash generalised | 1 (4%) | - | - |
| Rash pruritic | - | 1 (4%) | - |
| Thrombocytopenia | - | - | 1 (4%) |
| Thrombosis | - | 1 (4%) | - |
| Vitreous floaters | 1 (4%) | - | - |
| White blood cell count decreased | - | 1 (4%) | - |

## DISCUSSION

Pembrolizumab appears to elicit significant clinical activity with durable responses and a manageable safety and toxicity profile in patients with PD-L1–positive MPM. There was no treatment-related mortality and no discontinuations attributable to treatment-related AEs. Three of the reported responses were observed at the first imaging assessment. Three of the reported responses were ongoing and one patient continued to derive clinical benefit at the time of data cutoff, suggesting durability of response.

The patient population in this study was generally similar to those reported elsewhere in a second-line setting. However, unlike most of those other studies[9–14], this population did not include patients with severely disabling disease (i.e. only ECOG 0 or 1), and unique to the present study was the entry requirement for PD-L1–expressing tumours. Although comparison with other studies is difficult because of heterogeneity with respect to patient numbers, study entry criteria, study design, and history of patient treatment, the OR we report is within the range (7-20%) reported elsewhere for second-line options in general.[9–14] Of particular note is the effect of pembrolizumab on survival; the median OS we report is among the longest for second-line therapy in this patient population (5·7-10·9 months),[9–14] as is the median DOR (2·8-8·0 months).[10,12] Patients who do not receive second-line therapy have median survival of 6–7 months.[26,27]

In addition to pembrolizumab, another PD-1 pathway inhibitor investigated in MPM is the anti–PD-L1 antibody, avelumab. Recent results from the phase 1b JAVELIN study[30] investigating the safety and efficacy of avelumab in 53 patients with unresectable pleural or peritoneal mesothelioma who progressed after platinum-pemetrexed–containing therapy were promising.[28] The unconfirmed OR was 9·4% (all partial responses), median PFS was 17·1 weeks (95% CI 6·1–30·1) and toxicities were manageable.[30]

Safety and efficacy results from two investigator-sponsored phase 2 studies of the anti–CTLA-4 antibody tremelimumab were promising.[31,32] However, reports from the expanded phase 2b, double-blind, placebo-controlled DETERMINE study[29] did not show efficacy of tremelimumab as second-line or third-line therapy versus placebo in 571 patients with unresectable pleural or peritoneal mesothelioma. An ongoing phase 2 study (NCT02588131) is investigating tremelimumab in combination with the anti-PD-L1 antibody durvalumab in patients with malignant mesothelioma.

Angiogenesis inhibitors, in particular bevacizumab, have also demonstrated anti-tumour activity in MPM.[33,34] In an open-label, phase 3 trial, addition of bevacizumab to standard of care (pemetrexed plus cisplatin) in the first-line setting for patients with MPM improved OS compared with standard care alone (median 19 months vs 16 months). This treatment was associated with a high, but manageable, level of toxicity, with 71% of those receiving bevacizumab reporting grade 3-4 AEs compared with 62% of those who did not.[34]

Because the timing between initial pathological diagnosis and first dose of pembrolizumab and PFS from first diagnosis was not collected, there is no information regarding the natural history of the MPM tumours in these patients. Furthermore, modified MPM RECIST criteria were not used to assess disease progression for the primary analysis. Regardless, the depth and the durability of response assure the activity of pembrolizumab seen in these patients. In particular, the long follow-up and the durability of the responses seen in these patients argue against the survival expectation just from the natural history of MPM.

## CONCLUSION

The results from this phase 1b KEYNOTE-028 trial, showing clinical benefit in a proportion of MPM patients with PD-L1–positive tumours, indicate that further evaluation of pembrolizumab in malignant mesothelioma is warranted, and several phase 2 trials have been initiated. KEYNOTE-158 (NCT02628067) is an ongoing, multinational, phase 2 basket trial designed to assess biomarkers predictive of response to pembrolizumab (200 mg every 3 weeks) in several rare, advanced, solid tumors including MPM, not limited by tumour PD-L1 expression. Another trial (KEYNOTE-139; NCT02399371) is assessing the activity of fixed-dose pembrolizumab (200 mg every 3 weeks) as a second-line therapy for advanced malignant mesothelioma. Additionally, a third phase 2 active-comparator trial (NCT02784171) will explore the efficacy of first-line therapy of pembrolizumab versus either cisplatin and pemetrexed or the pembrolizumab/cisplatin/pemetrexed combination for advanced malignant mesothelioma. These ongoing studies use the updated dose of 200 mg every three weeks for pembrolizumab. Although weight-based dosing was used in the current study, population pharmacokinetics and exposure–response modelling have since shown that a fixed dose of 200 mg every three weeks would provide a similar exposure distribution to a 2 mg/kg dose delivered every three weeks.[35]

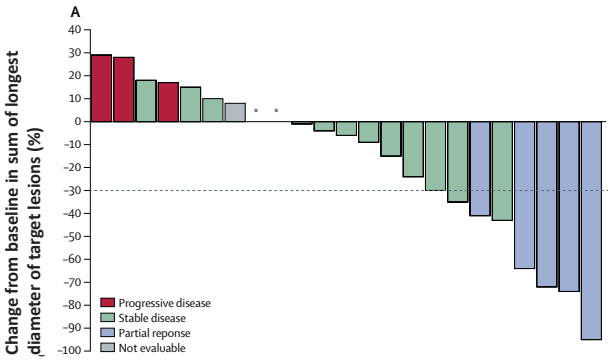
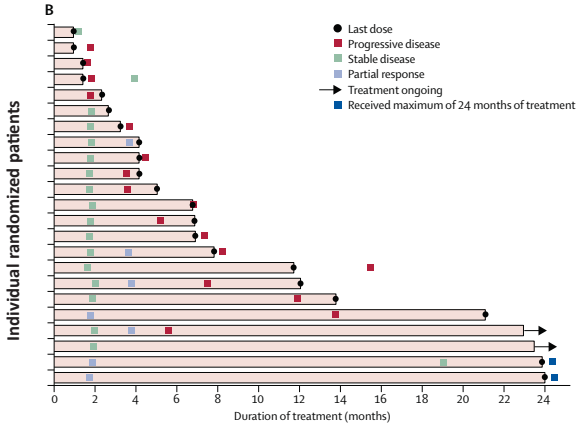



**Figure 2**. Tumor response to pembrolizumab according to RECIST (version 1.1).

**(A)** Maximum percentage changes from baseline in target lesions (RECIST v1.1, investigator assessed). Bar length represents change in target lesion size. Bar colour is best overall response. Dotted line is threshold for response. Two patients were listed as no assessment. *Indicates no change in size: one of these patients had a best overall response of stable disease, the other had partial response.




**(B)** Treatment exposure and response duration (RECIST v1.1, investigator assessed). The length of each bar corresponds to the duration of treatment for each patient. Response symbols represent the time first reported and not best overall response. A post-baseline tumour assessment was not available for two patients.

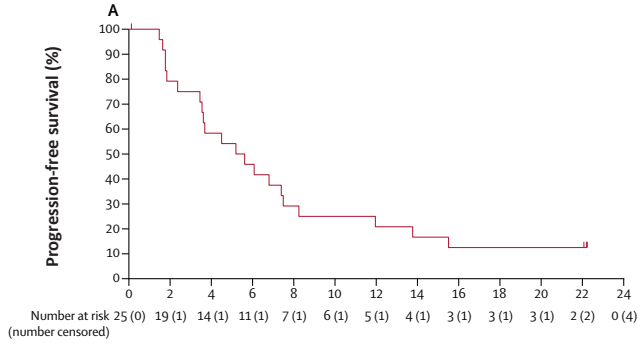
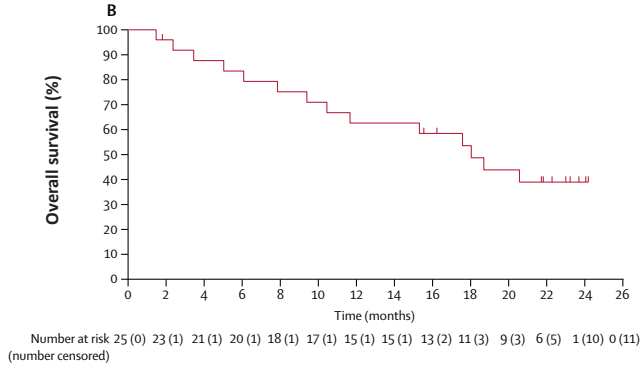



**Figure 3**. Kaplan-Meier curves of progression-free survival and overall survival.

**(A)** Progression-free survival (investigator-assessed).




**(B)** Overall survival.

Our results show that pembrolizumab might be safe for the treatment of MPM, with encouraging signs of anti-tumour activity in this patient population. Further study in larger, randomised trials is required to establish its position in the treatment arsenal for the larger MPM population.

## RESEARCH IN CONTEXT

### Evidence before this study

We searched PubMed on October 20, 2016, using the following terms: "PD-1 OR PD-L1 OR MK-3475 OR pembrolizumab OR nivolumab OR atezolizumab OR durvalumab OR avelumab" AND "mesothelioma". The search was not limited by date or language. We also searched the abstracts for the 2015 and 2016 American Society of Clinical Oncology Annual Meetings, the 2015 European Cancer Congress, the 2016 European Society for Medical Oncology Congress, the 2016 International Mesothelioma Interest Group meeting, and the 2015 World Conference for Lung Cancer using the same search terms. The literature review indicated that there were few studies of immune checkpoint inhibitors in patients with malignant pleural mesothelioma (MPM), despite evidence of PD-L1 expression in a subset of patients with MPM.

### Added value of this study

Results of the MPM cohort of the KEYNOTE-028 basket study suggest that pembrolizumab is a suitable treatment option for patients with MPM, with tolerable safety, an indication of clinical efficacy (ORR=20%, clinical benefit rate=40%), and substantial duration of response (median=12 months).

### Implications of all the available evidence

Our data suggest that additional studies of pembrolizumab in MPM are warranted. Interim phase 2 data were presented by Kindler et al. at the 2016 World Conference for Lung Cancer (KEYNOTE-139; ClinicalTrials.gov, NCT02399371), and KEYNOTE-158 (NCT02628067) is a phase 2 basket study that includes patients with MPM and is designed to evaluate biomarkers predictive of response to pembrolizumab as second-line therapy; there is also a phase 2 study exploring pembrolizumab as a first-line agent in comparison with standard of care cisplatin/pemetrexed chemotherapy (NCT02784171).

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# Chapter 4

# *BRAF* status as predictive biomarker

## Chapter 4.1

**_BRAF_ mutations as predictive marker for response to anti-EGFR monoclonal antibodies**

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**ABSTRACT**

Recently, the American Society of Clinical Oncology (ASCO) and European Society for Medical Oncology (ESMO) recommended that patients with epidermal growth factor receptor (EGFR) expressing metastatic colorectal cancer (mCRC) could be treated with anti-EGFR monoclonal antibodies (mAbs) cetuximab and panitumumab only in absence of Rat-Sarcoma (*RAS)* mutations[1,2]. In addition to the previously established biomarker Kirsten rat sarcoma viral oncogene homolog (*KRAS)* exon 2, cumulative evidence shows that also patients whose tumors harbor *KRAS* exon 3 or 4 and neuroblastoma rat-sarcoma viral oncogene homolog (NRAS) exon 2, 3 and 4 mutations are found unlikely to benefit from anti-EGFR treatment.

In line with the resistance of *RAS* mutated (mt) tumors, treatment response in *BRAF* mutated tumors may also be altered given their important role in the EGFR signaling pathway. However, *BRAF* is not recommended as predictive biomarker yet because the evidence for the impact of *BRAF* mutations on treatment outcome is considered insufficient.

This article summarizes the evidence for the impact of *BRAF* mutations on treatment outcome of anti-EGFR mAbs. Based on a review of literature, eight meta-analyses were included that consistently show that patients with *BRAF* mutations have a lack of treatment benefit of anti-EGFR mAbs. After discussing the quality and quantity of available evidence, we conclude that evidence is stronger than suggested by ESMO and ASCO. Additionally, we highlight that the quality of evidence for *BRAF* is even higher than for extended *RAS* as a biomarker. We therefore advise ESMO and ASCO to reconsider *BRAF* status as a predictive biomarker for response.

## INTRODUCTION

The Ras-Raf-MEK-ERK (MAPK) pathway plays a pivotal role in the regulation of cell proliferation, survival and differentiation. Constitutive activation of this pathway is frequently observed in human cancers and is associated with high rates of cancer cell proliferation. Within the MAPK pathway, Ras, Raf, MEK and ERK are key proteins in signal transduction. In tumor cells, the MAPK pathway is often constitutively activated by gain-of-function mutations in one of the signaling proteins including but not limited to Ras and Raf. In colorectal cancer (CRC), activation of the MAPK pathway is often a result of mutations in the Ras family protein Kirsten rat sarcoma viral oncogene homolog (*KRAS*) which are found in 40% of the patients[3,4]. Mutations occur most frequently in exon 2 (36%), and less frequently in exons 3 (2%) and 4 (2%). In addition to *KRAS*, neuroblastoma rat sarcoma viral oncogene homolog (*NRAS*) mutations occur in about 3%[3,4]. KRAS and NRAS are very closely related although their biological role is slightly different. Whereas functional KRAS is essential for cell survival, NRAS is not required. Therefore, KRAS gain-of-function mutations may have a larger impact on tumor growth and proliferation compared with NRAS mutations[5].

The first effector protein of Ras is Raf, comprising c-RAF1, BRAF and ARAF. Of these, BRAF has the most important biological function and is also most frequently mutated[6]. BRAF and RAS mutations are mutually exclusive which highlights their functional importance[7]. Gain-of-function mutations in exon 15 result in the BRAFV600E variant in about 10% of the CRC population and induce constitutive MAPK-pathway activation[5,8]. Other mutations that occur less frequently include the variants G469V (<0.1%), D594G (<0.3%) and K601E (unknown frequency)[6]. All mutations lead to constitutive activation of downstream proteins within the MAPK-pathway independent of upstream activation signals, yet the p.V600E variant is the strongest activator[8,9]. *BRAF* mutations in CRC occur most frequently in tumors originating from the appendix and the ascending and transverse colon, defined as right-sided tumors[10–12].

In up to 90% of colorectal tumors, epidermal growth factor receptor (EGFR) is overexpressed which renders EGFR an attractive drug target[13–16]. Upon binding of its ligands, including epidermal growth factor (EGF), betacellulin, epiregulin and neuregulins, cell proliferation and growth are induced primarily through the MAPK and PI3K/AKT signaling pathways (figure 1)[17]. Cetuximab (Erbitux®[14,18]) and panitumumab (Vectibix®[15,19]) are anti-EGFR mAbs that exert their anti-tumor effect through inhibition of EGFR signaling. Both drugs are registered for the treatment of metastatic EGFR expressing CRC (mCRC) after failure of first and/or second line therapies. In line with the biological mechanism, several trials showed that the effects of anti-EGFR treatment are decreased when mutations downstream of EGFR are present that cause MAPK-pathway activation independent of EGFR signaling such as mutations in KRAS (in 40%) and BRAF (in 10%)[1,3–5,20]. It is now generally accepted that mutations in *KRAS* exon 2 diminish treatment response when anti-EGFR mAbs are given as a single agent or combined with chemotherapy[1,14,15,21]. More recently, several retrospective analyses showed that not only *KRAS* exon 2 mutations, but also *KRAS* exon 3,4 and *NRAS* exon 2, 3 and 4 mutations are predictive biomarkers[1,2,7,22–27]. Treatment guidelines for mCRC now recommend upfront *RAS* testing before start of anti-EGFR mAb therapy[1,2], in order to exclude patients with mutated *RAS* from therapy with these agents.

*BRAF* mutations could have comparable effects on anti-EGFR mAb treatment response as *RAS* mutations. BRAFV600E gain-of-function mutations comprise 80%-96% of all *BRAF* mutations and occur in about 10% of CRC patients[4,6,28]. Although several meta-analyses indicate that *BRAF* status may be a predictive biomarker for treatment efficacy[4,29–32], the use of *BRAF* status as a predictive biomarker is not recommended yet because evidence is considered less convincing than the evidence for *RAS* mutations[1,2].

This manuscript describes the evidence that is available for *BRAF* mutations as a predictive biomarker for response to anti-EGFR mAbs in mCRC. We will discuss the load and quality of clinical evidence for the impact of *BRAF* mutations on anti-EGFR mAb treatment outcomes, and argue why this can be considered convincing enough to include *BRAF* mutation status in the panel of upfront mutation tests in anti-EGFR mAb therapy.

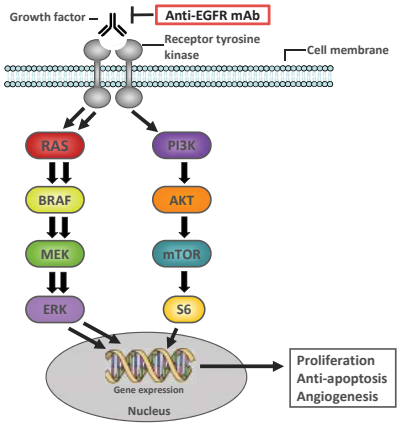


**Figure 1.** Schematic overview of the MAPK signaling pathway. Adapted with permission from van Geel et al[66].

**Evidence for *BRAF* mutations as predictive biomarker**

A PubMed search was performed to collect meta-analyses that included data of *BRAF* mutated (mt) patients and *BRAF* wildtype (wt) patients and survival outcome of treatment with the anti-EGFR mAbs cetuximab or panitumumab using the following terms (molecular testing OR mutation) AND (BRAF OR RAF) AND survival AND EGFR AND 'colorectal cancer' AND meta-analysis (full methods available in Supplemental Material 1). Eight meta-analyses were identified that report on the overall response rate (ORR), progression-free survival (PFS) or overall survival (OS) of *BRAF*mt patients treated with anti-EGFR mAbs cetuximab or panitumumab as single agents or combined with chemotherapy. Four of these were considered high-quality reviews and only these will be extensively discussed in this section[4,29–31]. The results of all meta-analyses are summarized in table 1.

De Roock *et al.*[4] comprehensively analyzed the relationship between different pathway mutations and treatment response and survival in mCRC patients treated with cetuximab combined with chemotherapy. The authors collected tumor samples and clinical data from 11 European investigators who had published data on cetuximab treated mCRC patients. Finally, 761 tumor samples were analyzed for *BRAF* status (screened for the mutations p.D594G, p.V600E, p.V600M and p.K601E). In 36 patients, a *BRAF* mutation was found, being mostly p.V600E (n=35) and one p.D594G. In a selection of patients without *KRAS* mutations, it was found that *BRAF*mt patients (n=24)) had a significantly lower ORR (8.3%) compared with *BRAF*wt patients (n=326, ORR 38%; odds ratio {OR} 0.15 [95% confidence interval {CI} 0.02-0.51]) and shorter PFS (hazard ratio {HR} 3.74 [95%CI 2.44-5.75]) and OS (HR 3.03 [95%CI 1.98-4.63]). The association between disease control and *BRAF* status was significant in multivariate analysis (adjusted OR *BRAF*mt vs. *BRAF*wt 0.059; $p$ <0.0001), as was the association with *KRAS, NRAS* and *PIK3CA* exon 20. Still, two out of 24 patients had a response to treatment despite *BRAF* mutations. The authors report that one of these had a p.D594G mutation that leads to weaker activation of the MAPK pathway compared with p.V600E mutations[33]. The other responder had a low copy number of *BRAFV600E* mutated genes that may explain the sensitivity to cetuximab. The authors conclude that the response rate of 24.4% in an unselected population could be increased to 36.3% in a *KRAS* wildtype population and further to 38.4% in *KRAS* and *BRAF* wildtype patients[4]. Another 1.5% ORR improvement could be achieved by *NRAS* testing according to their results. This study highlighted the importance of *BRAF* in addition to *KRAS* status in treatment with anti-EGFR mAbs. Several meta-analyses have been performed to confirm the findings of De Roock *et al.*

Pietrantonio *et al.*[31] performed a meta-analysis of randomized clinical trials (RCTs) to examine the effect of anti-EGFR mAbs on PFS, OS and ORR in *BRAF*mt/*KRAS*wt advanced CRC. Nine phase III trials and one phase II trial were included that compared anti-EGFR mAbs as monotherapy or added to chemotherapy with chemotherapy or best supportive care (BSC) in advanced *KRAS*wt CRC. In total, these comprised 6,256 patients on first-line (six trials) and second-line treatment (two trials) or who were chemo-refractory (two trials). The authors show that patients with *BRAF*mt CRC (n=469) do not have a significant benefit in PFS (HR PFS benefit 0.88 [95%CI 0.67-1.14]), OS (HR OS 0.91 [95%CI 0.62-1.34]) or ORR (OR 1.31 [95%CI 0.83-2.08]) from treatment with anti-EGFR mAbs. All mutations comprised p.V600E mutations except for 21 patients (13%) in the trial from Smith *et al.* who had the p.D594G mutation[34]. The response rate of patients with *BRAF* mutations varied from 10.8% to 52.2% on anti-EGFR mAbs compared with 6.4% to 40% on chemotherapy. Based on these results, *BRAF*mt patients seem to have modest responses to anti-EGFR mAbs yet overall, a significant response rate and survival benefit is lacking in this population[31]. A drawback of this meta-analysis is that a comparison with *BRAF*wt patients has not been made.

Rowland *et al.*[30] reviewed RCTs that evaluated the effect of *BRAF* mutations on treatment benefit (OS and PFS) from anti-EGFR mAbs for *KRAS* exon 2 and 3 wildtype mCRC. All of the included trials have also been reviewed by Pietrantonio *et al.*[31]. However, Rowland *et al.* excluded the trials by Tveit *et al.*[35] and Stintzing *et al.*[24], probably because the former did not provide data on PFS and OS and the latter had bevacizumab with FOLFIRI as control treatment instead which did not meet the inclusion criteria. The review by Rowland *et al.* thus differs from Pietrantonio *et al.* by inclusion criteria but moreover by their statistical tests[30,31].

Seven articles, covering eight RCTs, were included in which 3,168 *KRAS*wt patients were treated with cetuximab or panitumumab (four studies each) added to chemotherapy or with chemotherapy alone. About 11% of the tumors harbored a *BRAF* mutation (n=351), of which 94% (n=330) were p.V600E mutations and 6% (n=21) were p.D594G mutations[34]. Rowland *et al.* not only reported outcomes for the *BRAF*mt subgroup but also compared *BRAF*mt patients with *BRAF*wt patients. Results show a lack of PFS benefit in the *BRAF*mt group (HR PFS benefit 0.86 [95%CI 0.61-1.21]) whereas patients with *BRAF*wt had significant benefit (HR PFS benefit 0.62 [95%CI 0.50-0.77]) from addition of anti-EGFR mAbs to chemotherapy. The interaction test (PFS HR *BRAF*mt/PFS HR *BRAF*wt) showed a close to significant difference ($p$=0.07). For OS, *BRAF*mt patients (HR 0.97 [95%CI 0.67-1.41] also had no benefit whereas *BRAF*wt patients (HR 0.81 [95%CI 0.70-0.95]) had significantly improved OS. The interaction test

**Table 1.** Overview of meta-analyses which show hazard ratios for progression free survival (PFS) and overall survival (OS) and odds ratios for overall response rate (ORR) on anti-EGFR mAb therapy by *BRAF* status for the *KRAS*wt group and (°) for the *KRAS* unselected group. Four meta-analyses included only or primarily randomized clinical trials[29–31,38], three included retrospective and prospective studies[32,36,37].
*Abbreviations: n, number of patients; n BRAFmt, number of patients with a BRAF mutation; ^, based on re-calculation performed by the authors of this proposal; n.d., not described; HR, hazard ratio; OR, odds ratio; mt, mutated; wt, wildtype.*

| | ***BRAF*mt** | | | ***BRAF*wt** | | | ˣ ***BRAF*mt/*BRAF*wt**<br>ʸ ***BRAF*wt/*BRAF*mt** | | |
|---|---|---|---|---|---|---|---|---|---|
| Study (n/n *BRAF*mt) | PFS | OS | ORR | PFS | OS | ORR | PFS | OS | ORR |
| De Roockˣ (761/36)[4] | | | | | | | 3.74<br>[2.44-5.75] | 3.03<br>[1.98-4.63] | 0.15<br>[0.02-0.51] |
| Pietrantonio (6,256/469)[31] | 0.88<br>[0.67-1.14] | 0.91<br>[0.62-1.34] | 1.31<br>[0.83-2.08] | | | | | | |
| Rowlandˣ (3,186/351)[30] | 0.86<br>[0.61-1.21] | 0.97<br>[0.67-1.41] | n.d. | 0.62<br>[0.50-0.77] | 0.81<br>[0.70-0.95] | n.d. | 1.39^<br>[0.92-2.08] | 1.19^<br>[0.80-1.78] | n.d. |
| Therkildsenˣ | | | | | | | 2.95<br>[1.89-4.61] | 2.52<br>[1.39-4.56] | 0.29<br>[0.16-0.54] |
| Yuanʸ(4,616/343)[32] | | | | | | | 0.29<br>[0.19-0.43] | 0.26<br>[0.20-0.36] | 0.31<br>[0.18-0.53] |
| Xuˣ (2,875/246)[36] | | | | | | | 2.41<br>[1.23-4.71] | 2.74°<br>[2.31-3.52] | 0.26<br>[0.07-0.98] |
| Wangˣ (1,352/74)[37] | | | | | | | 2.78<br>[1.62-4.76] | 2.54<br>[1.93-3.32] | 0.27<br>[0.10-0.70] |
| Cuiˣ (1,245/126)[38] | | | | | | | n.d. | n.d. | 0.43<br>[0.16-0.75] |

for OS was not significant (*p*=0.43*)*. For both PFS and OS, the difference between *BRAF*mt and *BRAF*wt patients was bigger in the second-line setting showing a very strong trend towards significance (interaction test PFS *p*=0.05; OS *p*=0.38). The authors conclude that based on the non-significant interaction test values, the effect of *BRAF* mutations on PFS and OS cannot be confirmed[30].

However, it is of great importance to note that the interaction test for PFS was very close to significance. To allow proper interpretation of the *p* value (0.07), confidence intervals of the interaction test value should be taken into account. However, these were not provided by the authors. Based on our re-estimation as described in the Supplemental Material 1 - Methods, the 95% confidence interval for the interaction on PFS should range from 0.92 to 2.08. This underscores that there is a high chance that anti-EGFR mAbs have a different effect on PFS in *BRAF*mt patients. To summarize, these results confirm a significant lack of PFS benefit of anti-EGFR mAb treatment in *BRAF*mt patients, which is relevantly though not significantly different from the *BRAF*wt patients.

Another meta-analysis was performed by Therkildsen *et al.*[29], who reviewed the impact of alterations in *KRAS* other than exon 2, *NRAS, BRAF*, *PIK3CA* and *PTEN* on clinical benefit of anti-EGFR treatment combined with chemotherapy in *KRAS* exon 2 wt patients in 21 RCTs and one non-randomized trial. Among 1,267 patients treated with cetuximab or panitumumab in first to greater than fourth setting mostly, *BRAF* mutations were detected in 123 patients, all of which were p.V600E except for one p.K601E variant. *BRAF*mt patients were found to have significantly lower ORR (OR ORR 0.29 [95%CI 0.16-0.54]) and shorter PFS (HR 2.95 [95%CI 1.89-4.61]) and OS (HR 2.52 [95%CI 1.39-4.56]) compared to *BRAF*wt patients. The authors also report that the response rate can be increased from on average 37.6% in *KRAS*wt selected patients to 39% in *KRAS*wt/*BRAF*wt selected patients.

In our opinion, these meta-analyses provide high-quality clinical evidence for the lack of efficacy of anti-EGFR mAb treatment on response and survival endpoints for patients with *BRAF* mutated tumors. Supporting evidence can be found in four meta-analyses that included survival endpoints based on retrospective studies mainly[32,36,37], or that included response rate as an endpoint only[38] (table 1).

**Evidence for *BRAF* compared to *RAS***

Initially, Erbitux® and Vectibix® were registered for patients with EGFR expressing mCRC only in presence of *KRAS* exon 2 wildtype. This was based on seven pivotal trials with cetuximab that show that the *KRAS* exon 2 mutated population had no benefit on primary endpoints ORR, PFS or OS[14,15,39]. This is supported by a review of five RCTs that was performed by ASCO[21]. The authors showed that all five trials comprising 2,095 patients consistently detected a lack of benefit from treatment with anti-EGFR mAbs in 720 patients with *KRAS* exon 2 mutations in terms of PFS and ORR (table 2) whereas *KRAS* exon 2 wildtype patients did have significant benefit.

**Table 2.** Summary of the results from five randomized clinical trials which report on the effects of *KRAS* exon 2 mutations, referred to in American Society of Clinical Oncology's clinical opinion update 2009[21]. *Abbreviations: FOLFIRI , 5-fluorouracil, folinic acid, irinotecan; FOLFOX, 5-fluorouracil, folinic acid, oxaliplatin; CAPOX, capecitabine, oxaliplatin; BSC, best supportive care; HR, hazard ratio; OR, odds ratio; ORR, overall response rate; OS, overall survival; PFS, progression free survival; B, bevazicumab; n.d., not described; KRAS, Kirsten rat sarcoma viral oncogene homolog; *p<0.05.*

| | | ***KRAS* exon 2 wildtype** | | ***KRAS* exon 2 mutation** | |
|---|---|---|---|---|---|
| | Treatment | PFS (months) | ORR | PFS (months) | ORR |
| Van Cutsem[22] (n=540) | Cetuximab + FOLFIRI | 9.9 | 59.3 | 7.6 | 36.2 |
| | vs. FOLFIRI | 8.7 | 43.2 | 9.1 | 40.2 |
| | **HR/OR** | 0.68* | 1.37* | 1.07 | 0.9 |
| Bokemeyer[13] (n=233) | Cetuximab + FOLFOX | 7.7 | 60.7 | 5.5 | 32.7 |
| | vs. FOLFOX | 7.2 | 37.0 | 8.6 | 48.9 |
| | **HR/OR** | 0.57* | 2.54* | 1.83* | 0.51 |
| Punt[67] (n=501) | Cetuximab + CAPOX-B | 10.5 | n.d. | 8.6 | n.d. |
| | vs. CAPOX-B | 10.7 | n.d. | 12.5 | n.d. |
| | **HR/OR** | n.d. | n.d. | n.d. | n.d. |
| Amado[39] (n=427) | Panitumumab | 12.3 | 17 | 7.4 | 0 |
| | vs. BSC | 7.3 | 0 | 7.3 | 0 |
| | **HR/OR** | 0.45* | n.d. | 0.99 | n.d. |
| Karapetis[57] (n=394) | Cetuximab | 3.7 | 12.8 | 1.8 | 1.2 |
| | vs. BSC | 1.9 | 0 | 1.8 | 0 |
| | **HR/OR** | 0.4* | n.d. | 0.99 | n.d. |

In October 2015, ASCO recommended to extend upfront testing of *KRAS* exon 2 with *KRAS* exon 3,4 and *NRAS* exon 2, 3 and 4[1]. In July 2016, the European Society for Medical Oncology (ESMO) supported this 'extended *RAS*' testing in their consensus guideline on the management of mCRC[2]. In Europe, these findings were incorporated in the product labels of Erbitux® and Vectibix®, which state that the benefit-risk ratio of treatment is negative for patients with *KRAS* or *NRAS* exon 2,3,4 mutations[18,19].

As supportive evidence for *KRAS* exon 2, 3 and 4 testing, ASCO referred to ten[40–49] meta-analyses and two[50,51] health technology assessment reports that reviewed 137 primary studies with 19,543 patients. Table 3 summarizes findings of these ten meta-analyses. In all studies, a statistically significant PFS benefit in patients without *KRAS* exon 2,3,4 mutations was found whereas benefit was not significant in *KRAS* exon 2, 3 and 4 mutants. The effects of *KRAS* mutations on OS were less consistent. Five out of the 13 trials detected no statistically significant difference in OS between *KRAS*mt and *KRAS*wt patients treated with anti-EGFR mAbs. This is mainly due to lack of consistent OS benefit when adding anti-EGFR mAbs to standard of care in the overall and *KRAS*wt population. In *KRAS*wt patients, OS benefit was detected in only six out of 13 trials[45–47,49–51].

In contrast to the evidence for *KRAS* exon 2, 3 and 4, only five articles report on the impact of *KRAS* exon 3,4 and *NRAS* mutations by itself[2,7,22,25–27](table 4). Although these five trials consistently show that patients with *RAS* mutations do not have significant treatment benefit, it should be noted that this is based on a small group with *RAS* mutations other than *KRAS* exon 2. The subgroup of *KRAS* exon 3, 4 and *NRAS* exon 2, 3 and 4 mutated patients comprises only 10-20% of the study populations which were 360 patients in total. To increase the power, most studies merge patients with any *RAS* mutation into one or two groups. This supports extended *RAS* testing, but does not provide evidence on the effect of *KRAS* exon 3,4 and *NRAS* mutations by itself. In addition, the detected lack of OS benefit in the *RAS*mt subgroup is of limited value in three out of five trials because the *RAS*wt group did not have OS benefit either and no significant interaction has been confirmed.

Despite these limitations, evidence was considered convincing enough by ASCO and ESMO to recommend upfront *KRAS* and *NRAS* exon 2,3 and 4 mutation testing, so that only patients whose tumors do not harbor mutations in these exons will be given anti-EGFR mAb therapy[1,2].

**Table 3.** Summary of the data from meta-analyses addressing the effect of KRAS exon 2,3,4 mutations referred to in ASCO's clinical opinion update 2015[1]. All meta-analyses included randomized clinical trials only, except for Qiu[46] and Dahabrabeh[41] *et al.* who included also retrospective and observational studies. *Abbreviations: P, panitumumab; C, cetuximab; SOC, standard of care; BSC, best supportive care; n, included population; n.d., not described; OS, overall survival; PFS, progression free survival; HR, hazard ratio, KRAS, Kirsten rat sarcoma viral oncogene homolog; [P], PFS in months; [O], OS in months.*

| | | ***KRAS* exon 2,3,4 wildtype** | | ***KRAS* exon 2,3,4 mutation** | |
|---|---|---|---|---|---|
| | Treatment | HR PFS | HR OS | HR PFS | HR OS |
| Adelstein[40] (n=8,924) | P/C vs. SOC or P/C + SOC vs. SOC<br>Interaction: *KRAS*wt/*KRAS*mt | 0.80 [0.64-0.99]<br>0.71 [0.57-0.90] | n.d. | 1.11 [0.97-1.27] | n.d. |
| Dahabreh[41] (n=1,945) | P/C +- SOC vs. BSC or SOC<br>Interaction: *KRAS*mt/*KRAS*wt | n.d. | n.d. | n.d.<br>2.22 [1.74-2.55] | n.d.<br>1.30 [0.95-1.78] |
| Lin[42] (n=5,325) | P/C +SOC vs. SOC | 0.66 [0.53-0.82] | n.d. | 1.07 [0.91-1.27] | n.d. |
| Loupakis[43] (n=6,609) | P/C+ SOC vs. SOC | 0.91 [0.84-0.99] | 0.95 [0.87-1.04] | 1.13 [1.03-1.25] | 1.04 [0.95-1.13] |
| Petrelli[44] (n=484) | P/C+ SOC vs. SOC | 0.68 [0.53-0.87] | 0.88 [0.65-1.20] | n.d. | n.d. |
| Petrelli[45] (n=3,254) | P/C+ SOC vs. SOC | 0.65 [0.51-0.83] | 0.84 [0.73-0.98] | n.d. | n.d. |
| Qiu[46] (n=2,188) | C + SOC vs. SOC<br>Interaction: *KRAS*mt/*KRAS*wt | 5.8[P]<br>1.94 [1.62-2.33] | 6.9[O]<br>2.17 [1.72-2.74] | 3.0[P] | 13.5[O] |
| Vale[47] (n=5,966) | P/C+ SOC vs. SOC<br>Interaction: *KRAS*wt/*KRAS*mt | 0.83 [0.76-0.90]<br>0.78 [0.68-0.89] | 0.89 [0.82-0.97]<br>1.04 [0.95-1.15] | 1.06 [0.96-1.17] | 1.04 [0.95-1.15] |
| Zhang[48] (n=2,912) | P/C+ SOC vs. SOC | 0.64 [0.50-0.84] | 0.84 [0.64-1.11] | 1.37 [0.81-2.31] | 1.03 [0.74-1.44] |
| Ibrahim[49] (n=2,115) | P + SOC vs. SOC | 0.58 [0.36-0.93] | 0.90 [0.76-1.05] | n.d. | n.d. |

**Table 4.** Overview of RCTs showing hazard ratios for progression free survival (PFS) and overall survival (OS) stratified by *RAS* status, referred to in the American Society of Clinical Oncology's clinical opinion updates of 2009 and 2015[1,21]. *Abbreviations: FOLFOX, folinic acid + fluorouracil + oxaliplatin; FOLFIRI, folinic acid + fluorouracil + irinotecan; n.d., not described; n, population evaluable for RAS status; P, panitumumab; C, cetuximab; B, bevacizumab; KRAS, Kirsten rat sarcoma viral oncogene homolog; NRAS, neuroblastoma rat sarcoma viral oncogene homolog; RAS, rat sarcoma viral oncogene homolog.*

| | | **No *RAS* mutation** | | ***KRAS* exon 2 mutation** | | ***KRAS* exon 2 wildtype, *KRAS* exon 3,4 or *NRAS* exon 2,3,4 mutations** | | **Any *RAS* mutation** | |
|---|---|---|---|---|---|---|---|---|---|
| Author | Treatment | PFS | OS | PFS | OS | PFS | OS | PFS | OS |
| Douillard[7] (n=1,060) | P + FOLFOX4 vs. FOLFOX4 | 0.72 [0.58-0.90] | 0.78 [0.62-0.99] | 1.29 [1.04-1.62] | 1.24 [0.98-1.57] | 1.28 [0.79-2.07] | 1.29 [0.79-2.1] | 1.31 [1.07-1.6] | 1.25 [1.02-1.55] |
| Van Cutsem[22] (n=639) | C+ FOLFIRI vs. FOLFIRI | 0.56 [0.41-0.76] | 0.69 [0.54-0.88] | n.d. | n.d. | 0.81 [0.39-1.67] | 1.22 [0.69-2.16] | 1.10 [0.85-1.42] | 1.05 [0.86-1.28] |
| Schwartzberg[27] (n=285) | P + mFOLFOX6 vs. B + mFOLFOX6 | 0.65 [0.44-0.96] | 0.63 [0.39-1.02] | n.d. | n.d. | 1.39 [0.73-2.64] | 0.41 [0.19-0.87] | n.d. | n.d. |
| Peeters[26] (n=1,014) | P + FOLFIRI vs. FOLFIRI | 0.70 [0.54-0.91] | 0.81 [0.63-1.03] | 0.85 [0.68-1.06] | 0.94 [0.76-1.15] | 0.89 [0.56-1.42] | 0.83 [0.53-1.29] | 0.86 [0.70-1.05] | 0.91 [0.76-1.10] |
| Bokemeyer[25] (n=118) | C + FOLFOX4 vs. FOLFOX4 | 0.53 [0.27-1.04] | 0.94 [0.56-1.56] | n.d. | n.d. | 0.77 [0.28-2.08] | 1.09 [0.44-2.68] | 1.54 [1.04-2.29] | 1.29 [0.91-1.84] |

## DISCUSSION

### Strong evidence for impact of *BRAF*mt on anti-EGFR mAb treatment outcome

ASCO's and ESMO's most recent guidelines for the treatment of mCRC posit that there is currently insufficient evidence to recommend *BRAF* mutations as a biomarker for response to anti-EGFR therapy[52]. ESMO's guideline refers to three clinical trials[53–55] and two meta-analyses[30,31] which show conflicting results. The authors of the guideline suggest that the evidence for *BRAF* mutations as a predictive biomarker for anti-EGFR therapy in later lines is accumulating but the role in earlier treatment lines is uncertain[2]. It is therefore recommended that *BRAF* status is used as a prognostic marker or as a selection tool for clinical trials only. Based on our literature review, the evidence for the use of *BRAF* as predictive marker may be stronger than suggested.

All eight meta-analyses that were reviewed, covering first-line and second-line settings (Supplemental Material 2), consistently show that *BRAF*mt patients do not have significant benefit from anti-EGFR mAbs in terms of ORR, PFS and OS[30,31] and when compared to *BRAF*wt patients, they have significantly less ORR, PFS and OS benefit (table 1)[4,29,32,36,37]. A significant interaction between *BRAF* and outcome has been confirmed in five meta-analyses[4,29,32,36,37]. Because both cetuximab and panitumumab were registered mainly based on PFS benefit[7,18,19,22,25–27], the detected lack of PFS benefit in *BRAF*mt patients should warrant the use in this population.

Only Rowland *et al.* reported a non-significant interaction between the *BRAF*wt and *BRAF*mt group on both PFS and OS, although the result for PFS was close to significant ($p$=0.07). This finding should be interpreted in context with the power to detect significant differences. Results of Rowland *et al.* are based on a group of 3,096 patients. Although this is one of the three most extensive meta-analyses, it provides a power of 19%[56], whereas a sample size of 6,500 patients is required to detect a significant interaction effect with a power of 80% (calculation provided in methods section)[30].

Overall, the load of strong clinical evidence for *BRAF* mutations as biomarker is based on a population of 628 patients with *BRAF* mutations[4,29,31]. In comparison, the evidence for *KRAS* exon 3, 4 and *NRAS* exon 2, 3 and 4 comes from only five studies with 360 patients with *RAS* mutations other than *KRAS* exon 2[7,22]. The lack of treatment benefit in this group guided ASCO's and ESMO's clinical opinion on extended *RAS* testing, while a significant interaction between *KRAS* exon 3,4/*NRAS* exon 2,3,4 mutant and wildtype patients has not been confirmed. Based on this, the evidence for the impact of *BRAF* mutations on efficacy of anti-EGFR mAb treatment as a single agent or combined with chemotherapy can be considered stronger than for *KRAS* and *NRAS* mutations.

### Risks of using *BRAF* as predictive marker

If upfront molecular testing of *BRAF* will be applied, it is expected that 10% of the mCRC population will be identified as *BRAF*mt and will be excluded from anti-EGFR mAb therapy. The most important risk of the use of *BRAF* as predictive biomarker lies in withholding *BRAF*mt patients from a potentially effective treatment with anti-EGFR mAbs. In *BRAF*mt patients, response rates of 8.3%[4] to 18%[36] have been reported compared with 38%[4] to 42.4%[36] in *BRAF*wt patients. Although a direct effect of anti-EGFR mAbs cannot be ruled out, the responses may also be induced by backbone chemotherapy, which was administered in the majority of trials and which induced response rates of 13-40%[31]. This is supported by the finding that responses rates in *BRAF*mt patients are higher when anti-EGFR mAbs are combined with chemotherapy (ORR 8.3-18%[4,36]) compared to monotherapy (ORR 1.2%[57]). As a comparison, it should be noted that also in patients with *KRAS* exon 2 mutations relevant response rates of 33-36%[13,54] have been observed on anti-EGFR treatment added to chemotherapy (table 2) as well as incidental responses on monotherapy[18]. Yet, this did not hinder implementation of *KRAS* as a biomarker.

Biologically, it is possible that *BRAF*mt tumors respond to anti-EGFR treatment due to tumor heterogeneity, low copy numbers and/or a varying potency of BRAF mutations to activate the MAPK pathway[8]. As an example, the p.V600E mutation is a strong pathway activator whereas the p.D594G is less activating and may still allow responses[58,59]. It is unlikely that this plays a big role in the study results as described in this review because the majority of patients had BRAFV600E mutations. However, it may explain specific cases of responders. Importantly, preclinical and clinical evidence shows that *BRAF* mutations may sensitize tumors to anti-EGFR treatment when combined with targeted agents such as BRAF and MEK inhibitors[60–62]. The potential of these combinations is currently studied in clinical trials[62,63]. The use of *BRAF* as predictive marker as discussed in this review therefore only applies to anti-EGFR monotherapy or combined with chemotherapy.

### Limitations

The robustness of evidence for *BRAF* mutations as predictive biomarker is limited by some factors. Firstly, compared to *KRAS*, *BRAF* mutations may have a less pronounced predictive effect. Whereas patients with

*KRAS* exon 2, 3 and 4 mutations have 10-30% higher risk of progression during treatment with anti-EGFR mAbs compared to control treatment (table 3, 4), the effect of *BRAF* mutations seems smaller. This is based on PFS HRs with a wider confidence interval in patients with *BRAF*mt on anti-EGFR therapy (table 1) compared with patients *KRAS* exon 2, 3 and 4 mutations (table 3). However, it should be noted that the evidence for extended *RAS* testing was based on groups in which patients with *KRAS* exon 3, 4 and *NRAS* exon 2, 3 and 4 mutations were merged. The effect of these mutations is even more convincing if also *KRAS* exon 2 mutations are included (any *RAS* mutation, table 4). Merging all *RAS* mutations is needed to improve the power of the study. However, when comparing the load of evidence for extended *RAS* to the load of evidence for *BRAF*, it should be taken into account that the group of *BRAF*mt patients is always analyzed separately resulting in a less evident result. Merging this group with *RAS*mt patients would improve the power, but the relevance is questionable because of the different biology of the mutations.

Secondly, the impact of patient selection by *BRAF* status on OS remains uncertain. Because even in *RAS*wt and *BRAF*wt patients, the OS benefit of anti-EGFR mAbs is not consistently confirmed among different studies (table 4), OS seems an unreliable endpoint to assess the predictive value of *BRAF* for outcomes on anti-EGFR therapy. Therefore, the effects on PFS should guide decision making instead of effects on OS. In addition, uncertainties about the predictive value of *BRAF* may be a result of other predictive biomarkers beyond *KRAS* and *BRAF*. Recent evidence highlights the importance of primary sidedness on CRC prognosis and response[12]. Right and left sided tumors have a different biological origin resulting in different molecular characteristics. While left-sided tumors are associated with EGFR overexpression, right-sided tumor more often carry *BRAF* mutations[10,12] and are associated with poorer response and a shorter survival independent of treatment[11,64]. Although sidedness has been identified as an independent biomarker[11], the association between sidedness, *KRAS* and *BRAF* status and response to anti-EGFR mAbs is still to be clarified.

Moreover, cumulating evidence shows that in absence of a confirmed *BRAF* mutation, a similar gene expression profile can be present referred to as *BRAF*-like tumors. *BRAF*-like tumors have comparable characteristics as *BRAF*mt tumors, leading to treatment resistance by constitutive MAPK-pathway activation independent of EGFR signaling. Future clinical validation studies should reveal whether the evidence for *BRAF* status as predictive biomarker could become stronger by including *BRAF*-like gene signatures[65].

## CONCLUSION

Recent guidelines recommend upfront extended *RAS* testing in mCRC patients in order to exclude patients with *KRAS* exon 2, 3 and 4 and *NRAS* exon 2, 3 and 4 mutations from therapy with anti-EGFR mAbs. As outlined in this review, the evidence for *BRAF* testing is of even higher level than the evidence for extended *RAS* testing. Across all studies, no ORR, PFS or OS benefit could be detected in any *BRAF*mt subgroup. Moreover, significant interactions of *BRAF* status with treatment outcome have been observed. This review highlights that despite limitations in power and effect size, the current evidence should be enough to draw conclusions. Based on consistent lack of benefit of anti-EGFR mAb therapy in *BRAF*mt patients, it is advised that anti-EGFR mAb therapy is excluded for these patients. The authors therefore encourage ASCO and ESMO to reconsider *BRAF* as a predictive biomarker as this will help in selecting patients for whom maximum treatment benefit is expected.

## DISCLOSURES

The authors have no conflicts of interest to disclose.

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**SUPPLEMENTAL MATERIAL 1 - METHODS**

A PubMed search was performed to collect meta-analyses that included data of *BRAF* mutated (mt) patients and *BRAF* wildtype (wt) patients and survival outcome of treatment with the anti-EGFR mAbs cetuximab or panitumumab using the following terms (molecular testing OR mutation) AND (BRAF OR RAF) AND survival AND EGFR AND 'colorectal cancer' AND meta-analysis. Of the 12 articles, ten were selected for review and two were excluded based on incomplete results or irrelevance. The same search without the term "meta-analysis" was performed to check if relevant randomized clinical trials were recently published and not included in the selected meta-analysis, focusing on publications after 2014. This was not the case. The quality of evidence was considered high if included articles in the meta-analyses were mainly randomized controlled trials (RCTs) and the results included survival outcome of *BRAF*mt vs. *BRAF*wt patients. Furthermore, the overlap of included trials in the selected meta-analyses was assessed (Appendix 1) and taken into consideration in the quality assessment. As a comparison, the evidence for *KRAS* and *NRAS* mutations was extracted from the American Society of Clinical Oncology (ASCO) guidelines of 2009 and 2016[1,2] and from the European Society for Medical Oncology (ESMO) guideline of 2016[3].

Statistical analyses were performed in R. The confidence interval for *BRAF*mt versus *BRAF*wt was calculated using the HR of 0.86 and 0.62 as provided by Rowland *et al.* using properties of two normal distributions and the following calculation:

```
a <- log(0.86)
b <- log(0.62)
a.low <- log(0.61)
b.low <- log(0.50)
se.a <- (a - a.low)/1.96
se.b <- (b - b.low)/1.96

logQ <- a - b
se.logQ <- sqrt(se.a ^ 2 + se.b ^ 2)
logQ.low <- logQ - 1.96 * se.logQ
logQ.high <- logQ + 1.96 * se.logQ

exp(logQ.low)
exp(logQ.high)
```

The required sample size to detect a significant interaction effect between *BRAF*wt and *BRAF*mt with a power of 80% was calculated with the PowerSurvEpi package assuming a hazard rate (HR) of 1.4 as a clinically relevant effect of *BRAF*mt[4], indicating a 40% increased risk of death during treatment with anti-EGFR mAbs in presence of a *BRAF* mutation, an event rate (death) of 50% during clinical studies[5], and 10% incidence of *BRAF* mutations[6].


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## SUPPLEMENTAL MATERIAL 2 - OVERVIEW OF INCLUDED TRIALS

**Supplemental Table -** Overview of clinical trials included in meta-analyses on *BRAF* mutations.
*Abbreviations: RCT, randomized clinical trial; n.r., not reported.*

| Author | Study design | Treatment line | Pietrantonio | Rowland | Therkildsen | Yuan | Xu | Wang | Cui |
|---|---|---|---|---|---|---|---|---|---|
| Bokemeyer 2011 (OPUS) | RCT | 1st | | | | | | | x |
| Bokemeyer 2012 (OPUS + CRYSTAL) | RCT | 1st | x | x | x | x | | x | |
| Benvenuti 2007 | Retrospective | 1st and ≥2nd | | | | x | x | | |
| Cappuzzo 2008 | Retrospective | ≥2nd | | | | x | x | | |
| De Roock 2010 | Retrospective | ≥2nd | | | x | x | x | x | |
| Di Nicolantonio 2008 | Retrospective | ≥2nd | | | | x | x | | |
| Douillard 2013 (PRIME) | RCT | 1st | x | x | x | | | | |
| Fornaro 2011 | Retrospective | ≥2nd | | | | x | x | | |
| Freeman 2008 | Retrospective | 1st and ≥2nd | | | | x | x | | |
| Gao 2011 | RCT | n.r. | | | x | | | | |
| Garcia 2011 | RCT | ≥2nd | | | x | | x | | |
| Inno 2011 | RCT | n.r. | | | x | | | | |
| Karapetis 2014 | RCT | Refractory | x | x | | | | | |
| Laurent-Puig 2009 | Retrospective | ≥2nd | | | | x | x | | |
| Loupakis 2009 | RCT | Irinotecan-refractory | | | x | x | x | x | |
| Modest 2012 (AIO-KRK0104) | RCT | 1st | | | x | x | x | x | x |
| Molinari 2009 | RCT | Refractory | | | x | x | x | | |
| Montagut 2010 | Retrospective | 1st and ≥2nd | | | | | x | | |
| Moroni 2005 | Retrospective | 1st and ≥2nd | | | | x | x | | |
| Park 2011 | RCT | ≥2nd | | | x | x | | x | |
| Peeters 2013 | RCT | Refractory | x | x | x | | | | |
| Peeters 2014 | RCT | 2nd | x | x | | | | | |
| Perrone 2009 | RCT | Irinotecan-refractory | | | x | x | x | | |
| Saridaki 2011 | RCT | ≥2nd | | | x | x | x | x | |
| Sartore Bianchi 2009 | Retrospective | 1st and ≥2nd | | | | x | x | | |
| Seymour 2013 (PICCOLO) | RCT | ≥2nd | x | x | | | | | |
| Smith 2013 | RCT | 1st | x | x | | | | | |
| Soeda 2013 | RCT | ≥3rd | | | x | | | | |
| Spindler 2011 | Prospective | 3rd | | | | x | | | |
| Stintzing 2014 (FIRE-3) | RCT | 1st | x | | | | | | |
| Tol 2010 | RCT | 1st | | | x | x | x | | |
| Tural 2013 | RCT | Refractory | | | x | | | | |
| Tveit 2011 (Nordic VII) | RCT | 1st | x | | | x | x | | x |
| Ulivi 2012 | Prospective | ≥2nd | | | x | | | x | |

# Chapter 5

# Conclusions and perspectives

## CONCLUSIONS AND PERSPECTIVES

The central theme of this thesis is targeted anticancer therapy. In seven out of ten chapters, we described the results of personalized treatment with targeted agents, using biomarker-based selection. By making these biomarker-based selections, we aim to treat only those patients who are most likely to benefit in terms of anti-tumor responses and prolonged (progression-free) survival.

As already touched upon in the preface of this thesis, this biomarker-based personalized treatment can only be successful if the right biomarkers are used, if the drug reaches its target, if the efficacy-toxicity balance is acceptable and if drugs are combined in a rational way.

### Biomarker selection

Biomarker selection is a complex and long-term process, as illustrated in chapter 4.1 on *BRAF* status as a predictive biomarker for response to anti-EGFR monoclonal antibodies (mAbs). The evidence for the selection of a biomarker is mostly derived from retrospective studies leading to suboptimal quality. Also, results are often conflicting and based on small populations. Altogether, uncertainties arise and most studies conclude that further research is needed before a biomarker can be used in practice. This relevantly delays decision making and personalized treatment. Hence, there is a clear need for high-quality evidence but also for a more decisive approach. The quality of evidence for biomarker selection can be improved by performing prospective studies with pre-defined biomarkers based on preclinical or clinical evidence. This is being brought into practice already, as illustrated by chapter 3.1 on a CEA-targeted interleukin-2 variant (CEA-IL2v) in patients with CEA-expressing tumors, in chapters 2.1, 2.2 and 2.3 on combinations of MEK and pan-HER inhibitors in *KRAS* mutant tumors and in chapters 3.2 and 3.3 on pembrolizumab in PD-L1 expressing tumors. In these trials, a broad range of data is collected such as DNA profiles, tumor biopsies and circulating immune or tumor cells, to facilitate evaluation and optimization of biomarker selection criteria. For example, in the studies in which we use *KRAS* as a biomarker, preclinical data indicate that besides *KRAS*, also other mutations in the mitogen-activated protein kinase pathway may predict response to treatment. The tumor biopsies and DNA/RNA profiles obtained from patients allow us to investigate the relevance of these preclinical biomarkers in the clinic. Another way to evaluate biomarkers is the use of a radioactively labeled drug as described in chapter 3.1. This revealed that CEA-expressing tumors indeed show a higher drug uptake which supports the use of CEA as a biomarker. Early evaluation of biomarker selection criteria in context with preclinical data should be encouraged for future early stage clinical trials to allow efficient optimization of biomarkers.

### Target engagement

In the same study in which we used a $^{89}$Zr-radioactive label to assess the drug disposition of CEA-IL2v, we obtained information on the engagement of the targets of this drug, being CEA and the interleukin-2 receptor. The use of radioactive labels is applicable to a broad range of novel agents for which the biodistribution is unknown and provides us with a better understanding of their biodistribution, exposure and target engagement. This $^{89}$Zr-labelling technique is specifically relevant for monoclonal antibodies. This is firstly due to its long half-life, which allows detection of labeled drug during at least 10 days[1]. Secondly, labelling provides a method for measuring the drug disposition of monoclonal antibodies, as this cannot be adequately described by standard methods, such as mass balance studies or pharmacokinetic analyses in plasma. An important hurdle in the use of radiolabeled compounds is the *in vivo* stability. Although several clinical trials confirmed the feasibility of $^{89}$Zr-labeled antibodies, it stays unclear to what extent the detection of the radiolabeled molecule represents the intact molecule. Liberation of $^{89}$Zr from the conjugation product interferes mainly with quantitative analyses in these studies, and tools to minimize and assess this are desired[2]. Clinical application of $^{89}$Zr-labeled drugs is limited by the radiation dose for patients, which is significantly higher than for clinically-used radiolabels such as $^{99}$Tm and $^{111}$In. At the same time, alternative labels are less suitable for immuno-positron emission tomography (PET) detection ($^{124}$I, $^{111}$In, $^{67}$Ga, $^{99m}$Tc) or are eliminated too fast ($^{64}$Cu, $^{86}$Y, $^{99m}$Tc) to allow imaging[1].

A focus on target engagement was also made in the studies with MEK and HER inhibitors in *KRAS* mutant tumors. In these trials, tumor biopsies were obtained to analyze the modulation of proteins involved in cell growth and proliferation. Alternatively, peripheral blood mononuclear cells (PBMCs) or circulating tumor cells (CTCs) can be used for these analyses. PBMCs and CTCs are easier and safer to obtain than tumor biopsies. However, they provide outcomes on surrogate endpoints only whereas the tumor tissue is of primary interest. Before and on-treatment analyses of the target proteins, in either material, can provide valuable information to support the determination of the recommended phase 2 dose and thus their use is highly recommended in the development of targeted therapies.

**Efficacy-toxicity balance**

A third important factor in drug development is the balance between efficacy and toxicity. This applies to all drugs, including targeted anticancer agents. This balance comprises the tolerability on an individual and group level, compared to the medical need or the outcomes of available therapies for that specific patient group.

For the development of CEA-IL2v for example, it has become clear that despite confirmed target engagement in CEA expressing tumors, monotherapy with CEA-IL2v is insufficient for robust antitumor effects. This is amongst others due to suboptimal long-term exposure, as described in this thesis, and to limited tolerability. In contrast, efficacy may be improved in combination with other agents such as anti-PD-1 antibodies[3]. The potential of this combination was based on upregulation of PD-1 in on-treatment tumor biopsies, which highlights the importance of continuous biomarker analyses in early phase development. For anti-PD-1 antibodies, including pembrolizumab as described in chapter 3.2 and 3.3, the efficacy-toxicity balance seems predominantly positive which makes them attractive for single agent use but also in dual combinations. Although the number of responders is still low, about 30% at best as described in this thesis, the duration of response is remarkably long supporting the benefit of this treatment. At the same time, patients who are treated with anti-PD-1 antibodies are at risk for incidental but severe immune-related toxicities. This highlights again the need for biomarkers, to be able to select those patients for whom maximum treatment benefit is expected and to avoid exposure to these agents for patients who have a limited chance for response.

In the trials with a combination of MEK and HER inhibitors, the right balance between efficacy and toxicity is still to be found. Because three studies with the same concept (the same classes of drugs) were performed, class-effects of MEK and pan-HER inhibitors and intra-class differences can be assessed. In the continuous regimens, it seems that the efficacy of the lapatinib-trametinib combination is most pronounced. In this trial, lapatinib and trametinib could be escalated up to 50% of their single agent RP2Ds, whereas in the afatinib-selumetinib trial only 25% of the selumetinib RP2D and 50% of the afatinib RP2D could be administered. In the dacomitinib-PD combination only 33% of dacomitinib's RP2D could be given combined with 75% of the RP2D of PD-0325901. Furthermore, both lapatinib and trametinib have a long half-life which allows a relatively constant and continuous exposure to both agents. Unfortunately, this goes with the highest incidence of skin toxicity (100%), compared to 96% (dacomitinib-PD03325901) and 89% (afatinib-selumetinib) and with left-ventricular ejection fraction decreases in 19% which were not observed in the other trials. Other common class-effects of MEK and HER inhibitors were consistently observed in every trial and include skin rash, diarrhea, nausea, vomiting, mucositis, CK elevation and eye toxicities. Despite some responses, efficacy was overall disappointing compared to preclinical results and toxicity was dose-limiting in a substantial number of patients. Several hypotheses can be raised about the poor efficacy in these studies, such as inadequate dosing, lack of apoptosis, fast onset of resistance, intrinsic resistance or varying sensitivity of the different tumor types. Ideally, these hypotheses should have been tested in preclinical experiments before clinical application. On the contrary, extension of the preclinical phase significantly delays the development of potentially effective therapies. Moreover, even when the preclinical research phase is extended, there are discrepancies between the preclinical and clinical setting. For example, in preclinical experiments, Sun *et al.* reported apoptosis of colorectal cancer cells and non-small cell lung cancer cells after afatinib-selumetinib treatment[4]. Later, *in vivo* experiments showed that the extent of apoptosis may not be sufficient as posed by Verissimo *et al*[5]. In patients, the latter finding explains why we observed disease stabilization as best response in the majority of patients. Looking back, preclinical data on the impact of mutations beyond *KRAS* would have facilitated improved patient selection. However, a major extension of the preclinical phase would have been inefficient because results would not have predicted the toxicities, the resistance mechanisms after several weeks of treatment and do not take into account intratumoral heterogeneity. These aspects can only be evaluated in clinical trials including collection of tumor and blood samples. Currently, tumor samples are used for DNA and RNA sequencing which will hopefully reveal a relationship between genetic and protein expression profiles and response. These results may form the basis of refined patient selection and for strategies to overcome resistance. Furthermore, tolerability may be improved by adapting treatment regimens. However, the question is if the efficacy-toxicity balance will be sufficiently positive at tolerable doses to warrant further development. This question will hopefully be answered in due time.

The dose-finding process as described in previous paragraphs may be supported by pharmacokinetic and pharmacodynamic modelling techniques. In general, modelling and simulation can be used to explore not only population pharmacokinetics and pharmacodynamics, but also markers for treatment response and factors that influence the exposure or response to the drug and to guide dose-escalation. We explored the possibilities of using merged data from three clinical trials to support dose- and regimen finding, of which the results are

described in chapter 2.4. Although it is being recognized that modelling can facilitate clinical development, implementation is hindered by some factors such as a lack of expertise in hospitals and small-sized companies, limited external validity and insufficient model-transparency. Clear reporting of model-based approaches together with the right expertise will facilitate implementation and hereby help to improve biomarker selection, patient selection, and dose-finding.

**Combinations**

The clinical use of targeted anticancer agents constantly confronts us with resistance mechanisms. Upon inhibition of a signaling pathway, other signaling routes can be induced. This could be compared to a highway that is under construction; if the main road is blocked, traffic always finds other ways to reach the final destination. The use of combination therapies provides a way to suppress or overcome these resistance mechanisms. Although it may not be feasible to completely overcome this problem, it should be possible to delay it as much as possible, or even make use of the resistance that occurs as we did in the studies with MEK and HER inhibitors in *KRAS* mutant tumors[4]. One way or another, an important start is to understand the ways of resistance that cells use, which is already a big topic in preclinical and clinical research. On the basis of preclinical experiments, we can select rational drug combinations and identify any scheduled dependency of such combinations. For example, preclinical data showed that melanoma tumors may benefit from a drug-holiday of BRAF inhibitors, supporting an intermittent administration regimen in patients[6]. Also for combination therapies, information on the administration schedule can facilitate rational and efficient clinical studies although as indicated in the previous paragraph, clinical results may be different because of discrepancies between the *in vitro* and clinical setting such as tumor heterogeneity or pharmacokinetics. Other approaches, such as *in vivo* experiments, could reflect the clinical setting better, and hereby facilitate the translation from preclinic to clinic. More than resistance alone, also synergistic activity can be a reason to combine specific agents. For example, preclinical and clinical data suggest that the effect of immunotherapies such as anti-PD1 agents is potentially enhanced by radiotherapy or chemotherapy[7,8] which forms the basis of numerous ongoing clinical trials. Another advantage of using the synergistic activity of combinations, is that potentially lower doses of both agents can be used, allowing improved tolerability.

**Conclusion**

When combining optimal biomarker selection, adequate targeting, rational combinations and a positive efficacy-tolerability balance, then the chances for effective therapy are at highest. Based on several reviews of literature and on clinical experience from phase I trials, this thesis provides considerations for the early clinical development of novel anticancer agents, in order to accelerate improvements with a focus on selection of the right patients to treat, assessment of targeting and therapy effects, overcoming resistance mechanisms, and optimizing phase I trial design.

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# APPENDIX

**SUMMARY**

The research described in this thesis focuses on the early stage of clinical development of novel targeted anticancer agents. The early stage of clinical development includes phase I and II trials, drug-drug and food-drug interaction studies and proof-of-concept studies. This thesis focuses on phase I trials. Phase I trials often cover the first-in human application of a novel agent or a novel combination, in which the aim is to evaluate the safety and pharmacodynamic effects of the drug or drug-combination and to determine the recommended phase II dose and regimen. If feasible, phase I trials are followed by phase II, III and IV trials, which focus on the efficacy, pharmacodynamics and safety in larger populations and compare the novel drug to existing therapies. We describe the results of phase I trials of targeted anticancer agents and provide considerations for the early clinical development of novel anticancer agents, with a focus on selection of the right patient population, assessment of targeting and therapy effects, overcoming resistance mechanisms, the use of drug combinations and on optimization of phase I trial designs.

**Chapter 1** focuses on general aspects of phase I trials. **Chapter 1.1** is about the performance of phase I clinical trials with novel targeted anticancer agents in oncology. The primary aim of phase I studies is to determine the recommended phase II dose (RP2D). Traditionally, phase I trials are conducted according to a rule-based design, often a 3+3 design. This design mandates that three to six patients are treated at each dose-level, and the RP2D is the dose at which less than two out of six patients experience severe toxicities (defined as dose-limiting toxicities, DLTs). In the past years, novel designs have been introduced that use a model-based approach. Model-based designs require the development of a model predicting the dose-toxicity relationship, which can incorporate emerging safety data during conduct of the trial. Simulations showed superiority of model-based over rule-based designs in terms of a faster, more accurate determination of the RP2D with a reduction in overtoxicity. Based on a literature review, we compared the performance of rule-based and model-based designs in practice. Our results showed that model-based trials needed ten months less than rule-based trials to determine the RP2D. Moreover, more patients are treated at doses close to the RP2D with a potentially higher efficacy while safety was comparable to rule-based trials. Based on these findings, we encourage the use of model-based trials for future phase I studies. **Chapter 1.2** discusses the clinical relevance of anti-drug antibodies in oncology. In oncology, an increasing number of targeted anticancer agents and immunotherapies are of biological origin. These biological drugs may trigger immune responses which lead to the formation of antidrug antibodies (ADAs). ADAs are directed against immunogenic parts of the drug and may hereby affect efficacy and safety. This chapter focuses on the incidence and effects of ADAs and strategies to prevent ADA formation. Our results indicate that the majority (63%) of biological anticancer agents induces ADA formation, while the effects of these ADAs have only been explored in about 50% of the cases. Furthermore, inconsistent reporting and heterogeneity in detection methods complicate interpretation of the obtained results regarding ADA formation. In case ADAs are found to have relevant effects on the drug exposure or effects, then prevention methods may be considered. Potentially effective measures include adaptation of the treatment regimen and immunosuppressive treatment, although more research is needed to evaluate the feasibility in oncology.

**Chapter 2** describes the results of three phase I trials in which the same concept of combined inhibition of MEK and HER proteins is investigated. The potential of this combination was established in cell lines and mouse-models with colorectal cancer (CRC) and non-small cell lung cancer (NSCLC), in which cells with a mutation in the Kirsten rat sarcoma viral oncogene homolog (KRAS) protein showed relevant responses to these combinations whereas monotherapy with either agent was ineffective. This concept was translated into three different clinical trials in patients with *KRAS* mutant CRC, NSCLC or pancreatic cancer. In **chapter 2.1**, the combination of the MEK inhibitor selumetinib and the multiple-HER (pan-HER) inhibitor afatinib is investigated. **Chapter 2.2**, focuses on the combination of PD-0325901 and dacomitinib and **chapter 2.3** on the combination of trametinib and lapatinib. These phase I studies aim to determine the RP2D of the combination, and to investigate the safety and preliminary efficacy in NSCLC, CRC and pancreatic cancer. In all three studies, preliminary results show that toxicity limits the use of doses close to the single agent RP2Ds. Dose-limiting toxicities included diarrhea, skin toxicity, nausea/vomiting and liver enzyme elevation. The relevant toxicities at relatively low doses may be one of the reasons why efficacy in patients seems limited despite promising preclinical results. We did observe a difference in efficacy between the different tumor types, with a trend towards preferential activity in NSCLC. As a result, all three studies are now including NSCLC patients only to confirm this signal. Also, the search for the RP2D and optimal regimen is still ongoing. Currently, intermittent regimens are being explored to allow further dose-escalation with preserved tolerability. Among the three studies, it seems that the efficacy of the lapatinib-trametinib combination is most pronounced. Toxicities of the three two-drug combinations are comparable, with predominantly skin rash, diarrhea, nausea, vomiting, mucositis, CK elevation and eye toxicities. However, left-ventricular ejection fraction decreases occurred only on lapatinib-trametinib. The relationship between tox-

icity and exposure to MEK and pan-HER inhibitors was further explored in **Chapter 2.4**, in which a pharmacokinetic-toxicodynamic model was developed. Based on the observed toxicities in the combination trials, we questioned if the tolerability could be optimized by altering the ratio of MEK and HER inhibition. The first step towards answering this question is knowing how the MEK and HER inhibitors contribute to the observed DLTs. We therefore developed a model that incorporated merged data from the three trials, describing the quantitative relationship between exposure to MEK and HER inhibitors and the probability of developing a DLT. Also, we explored the relative contributions of MEK and HER inhibitors to DLT, and found that no distinction between the relative contributions of MEK and HER could be made. Furthermore, our model indicated that there is a relatively narrow dose range that can be used with an acceptable toxicity rate, and when exceeding these doses the risk of toxicity increases rapidly. This is in line with our clinical findings and may hinder dose-escalation to potentially more effective doses.

In **Chapter 3** several phase I studies with biological targeted anticancer agents are discussed. **Chapter 3.1** describes the biodistribution and tumor targeting (i.e. drug disposition) of the novel immunocytokine cergutuzumab amunaleukin (CEA-IL2v). This is an interleukin-2 variant (IL-2v) coupled to an antibody targeted to carcinogenic-embryonic antigen (CEA). This immunocytokine is ought to target tumor cells with CEA expression, where it can induce a local anti-tumor immune-response by binding to IL-2 receptors on lymphocytes. To study the biodistribution, CEA-IL2v was labeled with radioactive $^{89}$Zirconium ($^{89}$Zr), which allowed detection by $^{89}$Zr-positron emission tomography (PET). Subsequently, $^{89}$Zr-CEA-IL2v was administered to patients with solid tumors. Results of this trial revealed that the compound accumulates in liver, spleen and lymph nodes at all doses and accumulates in tumor mainly at doses >20 mg. In line with the design of the molecule, tumor targeting was more pronounced in CEA expressing tumors. Surprisingly, we found that the drug disposition changed during treatment. In the fourth cycle, liver accumulation was higher, while blood levels, spleen accumulation and tumor targeting were lower. We hypothesize that this is due to ADAs, saturation of IL-2 receptors and/or occupation of CEA binding sites in tumor. These results show that the targeted approach of CEA-IL2v works out in patients, although tumor targeting decreases during treatment. To overcome this, the optimal dosing strategy may include an on-treatment dose-escalation. Furthermore, this study confirms the feasibility of using $^{89}$Zr to study biodistribution of antibodies in patients and highlights the importance of on-treatment biodistribution studies to detect changes in drug behavior over time.

**Chapter 3.2** and **3.3** are about the preliminary efficacy of pembrolizumab, an anti-programmed death (PD)-1 antibody. The PD-1 receptor is expressed on various cells of the immune system such as natural killer cells and cytotoxic ($CD^{8+}$) T-cells and has immune-suppressing effects upon activation. By inhibiting PD-1 signaling, immune cells are stopped being suppressed, which leads to an enhanced immune response. Both chapters describe results from a phase I study in which the efficacy and safety of pembrolizumab was explored in 20 different tumor types. Results of other cohorts not described in this thesis are or will be published as separate papers. In **chapter 3.2** the merged results of all patients treated at the Netherlands Cancer Institute-Antoni van Leeuwenhoek hospital are described. Out of 17 patients with nine different tumor types, efficacy was observed in ten patients who had mesothelioma, nasopharyngeal cancer, ovarian cancer, carcinoid tumors or vulvar cancer. Major tumor regressions of 74% were observed in two patients, one with nasopharyngeal cancer and one with ovarian cancer with an acceptable safety profile. **Chapter 3.3** shows that also in patients with mesothelioma, promising responses were observed. In this chapter we present the results of all 25 patients included worldwide in this cohort, of whom 20% experienced a >30% decrease in tumor volume. These results highlight that responses to pembrolizumab can be durable. Responses lasted median 12 months in mesothelioma and overall survival was median 18 months, compared to an overall survival on other therapies that ranges from six to 11 months. The toxicity profile of pembrolizumab seems comparable in the different tumor types, with the most frequently observed toxicities being nausea and fatigue which did not lead to treatment interruption. Furthermore, patients are at risk of immune-related adverse events as a result of immune-activation by pembrolizumab, such as hypothyroidism, skin- and eye toxicities. Overall, the results of this trial support further investigation of pembrolizumab in mesothelioma, anal, vulvar, small cell lung, nasopharyngeal and cervical cancer, which is being brought into practice already.

**Chapter 4** describes the evidence for the use of *BRAF* status as a predictive marker for response to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (mAbs) in patients with metastatic CRC. Anti-EGFR mAbs are indicated for metastatic CRC with EGFR expression. It is already known that mutations in the KRAS protein lead to resistance to anti-EGFR treatment because these mutations activate the EGFR-signaling pathway independent of its ligand EGF. In line with this resistance mechanism, also mutations in the BRAF protein could and are suspected to lead to resistance. However, evidence has been considered conflicting and insufficient to truly conclude if *BRAF* mutations are predictive for the response to anti-EGFR mAb treatment. In our review,

we summarize the available evidence that describes the effect of *BRAF* mutations on treatment response. We found that none of the studies report a significant clinical benefit of anti-EFGR treatment in patients with a *BRAF* mutation, and conclude that this evidence should be sufficient to exclude patients with a *BRAF* mutation from treatment with anti-EGFR mAbs. Alternatively, these patients may benefit from chemotherapy only or from the combination of anti-EGFR mAbs and BRAF inhibitors which is currently in clinical trials and shows promising results, in contrast to monotherapy with anti-EGFR mAbs in this group of patients.

**NEDERLANDSE SAMENVATTING**

Dit proefschrift richt zich op vroeg-klinische ontwikkeling van nieuwe antikanker geneesmiddelen. Binnen de vroeg-klinische geneesmiddelontwikkeling ligt de focus van dit proefschrift op fase I studies. Deze studies richten zich vaak op de eerste klinische toepassing van nieuwe geneesmiddelen of van een nieuwe combinatie van geneesmiddelen, waarbij het doel is om de veiligheid, de eerste tekenen van effectiviteit en de juiste dosis met het juiste toedieningsschema voor verdere klinische toepassing te bepalen (maximaal tolereerbare dosis (MTD)). Als de fase I resultaten positief genoeg zijn, wordt de ontwikkeling vervolgd in fase II, fase III en fase IV studies. Hierin staan de effectiviteit en veiligheid in grotere patiëntgroepen centraal en worden deze vergeleken met de bestaande therapieën voor deze patiëntgroepen.

In dit proefschrift worden de resultaten beschreven van verschillende fase I studies met doelgerichte antikanker geneesmiddelen, en worden aanbevelingen gedaan voor de vroeg-klinische ontwikkeling van nieuwe antikanker geneesmiddelen. Hierbij ligt de focus op de selectie van de juiste patiëntgroepen, het monitoren van de effecten van therapie, manieren om resistentie te voorkomen of tegen te gaan, het gebruik van rationele combinaties van verschillende middelen en het optimaliseren van de opzet van fase I studies.

**Hoofdstuk 1** richt zich op algemene aspecten van fase I studies. **Hoofdstuk 1.1** gaat over de prestaties van fase I studies met doelgerichte antikanker geneesmiddelen. Prestaties omvatten in dit verband de snelheid van het uitvoeren van de studie, de veiligheid van de patiënten, en het aantal patiënten dat nodig is voor het bepalen van de MTD. Lange tijd werden fase I studies uitgevoerd volgens een 'rule-based' opzet, zoals de zogenaamde 3+3 methode. Volgens deze aanpak worden drie tot zes patiënten met een bepaalde dosis behandeld, en de MTD is de dosis waarbij minder dan twee van de zes patiënten ernstige bijwerkingen hebben (deze worden omschreven als dosis-limiterende bijwerkingen, DLTs). Afgelopen jaren zijn nieuwe methoden voor fase I studies ontwikkeld, die zich laten omschrijven als 'model-based' methoden. Voor model-based methoden dient een model te worden ontwikkeld die de relatie tussen dosis en toxiciteit beschrijft. In dit model kan informatie over de veiligheid van het middel die gedurende de studie verkregen wordt, geïncorporeerd worden waardoor dit model constant vernieuwd en verbeterd wordt. Simulatiestudies hebben laten zien dat model-based methoden een snellere manier bieden om de MTD met meer nauwkeurigheid vast te stellen terwijl minder patiënten ernstige bijwerkingen hebben door overdoseringen. Wij hebben een literatuuronderzoek gedaan om de prestaties van model-based fase I studies in de praktijk te vergelijken met die van rule-based fase I studies. Hierbij vonden wij dat met model-based studies gemiddeld tien maanden sneller een MTD bepaald kon worden. Ook werden meer mensen behandeld met doses rondom de MTD, wat een hogere kans geeft op een effectieve behandeling terwijl de veiligheid binnen model-based en rule-based studies vergelijkbaar was. Op basis van deze bevindingen kunnen wij het gebruik van model-based methodes aanbevelen voor toekomstige fase I studies met antikanker geneesmiddelen. **Hoofdstuk 1.2** beschrijft de klinische relevantie van antilichamen die gevormd worden tegen antikanker geneesmiddelen. Een toenemend aantal geneesmiddelen in de oncologie is van biologische oorsprong. Deze middelen kunnen een immuunreactie veroorzaken waarbij antilichamen tegen het geneesmiddel gevormd worden (anti-drug antilichamen, ADAs). Als ADAs aan het geneesmiddel binden, kunnen ze ervoor zorgen dat de werkzaamheid vermindert, of dat extra bijwerkingen optreden. Dit hoofdstuk richt zich op de incidentie en de gevolgen van ADAs en op strategieën om de vorming te voorkomen. Onze resultaten laten zien dat ADA vorming optreedt bij 63% van de geneesmiddelen van biologische oorsprong in de oncologie. De effecten van deze ADAs zijn echter in slechts 50% van de gevallen onderzocht. Ook zijn weinig details bekend over de manier waarop ADA bepalingen gedaan worden (zoals de soort ADAs, de analysemethoden en op welke tijdstippen de bepalingen gedaan zijn). Hierdoor is de klinische relevantie van ADAs in de oncologie moeilijk vast te stellen. Als ADAs gevonden worden die klinisch relevant zijn, die bijvoorbeeld in hoge concentraties aanwezig zijn en de effectiviteit of veiligheid beïnvloeden, dan zijn preventiemethoden gewenst. Potentieel kunnen aanpassingen van het doseerschema en behandeling met immuun-suppressieve middelen de vorming van ADAs tegengaan. Deze methoden worden (met wisselend effect) gebruikt in de reumatologie, maar hun toepassing in de oncologie behoeft nog nader onderzoek.

**Hoofdstuk 2** omvat de resultaten van drie verschillende fase I studies waarin het concept van gecombineerde MEK en HER remming in patiënten wordt onderzocht. In preklinisch onderzoek, in cellijnen en muismodellen met coloncarcinoom (CRC) en niet-kleincellig long carcinoom (NSCLC) met een mutatie in het Kirsten rat sarcoma viral oncogene homolog (KRAS) eiwit, bleek een combinatie van MEK en HER remmers een sterke remming van tumorgroei te geven, terwijl monotherapie met MEK of HER remmers ineffectief was. Vanwege deze veelbelovende resultaten werd dit concept toegepast in drie klinische studies bij patiënten met *KRAS* gemuteerd CRC, NSCLC of pancreas carcinoom. In **hoofdstuk 2.1** wordt de combinatie van de MEK remmer selumetinib en de HER remmer afatinib onderzocht. In **hoofdstuk 2.2** en **2.3** zijn dit de combinaties van respectievelijk PD-0325901 met dacomitinib en trametinib met lapatinib. Het doel van deze fase I studies is het vaststellen van de MTD van de combinaties, en het onderzoeken van de veiligheid en eerste tekenen van effectiviteit in NSCLC, CRC en pancreas carcinoom. In alle studies lijkt de toxiciteit van de combinatie het gebruik van effectieve doses te verhinderen. Patiënten ervaren frequent diarree, huidtoxiciteit, misselijkheid, braken of leverschade waardoor de therapie onderbroken moet worden. Dit kan een van de redenen zijn dat de klinische effectiviteit beperkt is ondanks goede preklinische resultaten. Van de drie studies lijkt de lapatinib-trametinib combinatie het meest effectief, terwijl de bijwerkingen erg vergelijkbaar zijn in de verschillende studies. De enige relevante uitzondering hierop is het optreden van afnames in de linker-ventrikel ejectie fractie, die uitsluitend voorkwam bij de lapatinib-trametinib combinatie. Ook zagen we dat patiënten met NSCLC meer baat lijken te hebben bij de MEK-HER combinatietherapie dan de patiënten met andere tumoren. Daarom richten de drie lopende studies zich nu uitsluitend nog op patiënten met NSCLC. In deze patiënten zoeken wij momenteel nog naar de MTD, waarbij we proberen om de doses verder te verhogen met behoud van tolerantie door het gebruik van intermitterende doseringsschema's.

De relatie tussen de dosis van MEK en HER remmers en de kans op het ontwikkelen van ernstige bijwerkingen werd nader onderzocht in **hoofdstuk 2.4**, waarin de ontwikkeling van een pharmacokinetisch-toxicodynamisch model wordt beschreven. Op basis van de bijwerkingen die gezien werden in de klinische studies, stelden wij de vraag of de tolerantie beter zou zijn bij een andere verhouding van MEK en HER remming. Om deze vraag te beantwoorden onderzochten wij eerst de relatie tussen MEK en HER remming en het optreden van ernstige, dosis-limiterende, bijwerkingen (DLTs). Hiertoe ontwikkelden wij een model waarin de data van de drie klinische studies gebruikt worden, die de kwantitatieve relatie tussen de blootstelling aan MEK en HER remmers en de kans op het ontwikkelen van DLTs beschrijft. Daarnaast onderzochten we de relatieve bijdragen van MEK en HER remmers op deze kans, waarbij bleek dat geen duidelijk onderscheid gemaakt kon worden tussen de bijdragen aan DLTs van de twee geneesmiddelgroepen. Dit model geeft ook aan dat er een sterke relatie is tussen blootstelling en toxiciteit, en de kans op bijwerkingen snel te hoog wordt bij verhogen van de doses. Dit blijkt ook uit onze klinische data, en kan het gebruik van effectieve doses mogelijk verhinderen.

**Hoofdstuk 3** omvat verschillende fase I studies met doelgerichte antikanker geneesmiddelen van biologische oorsprong. **Hoofdstuk 3.1** beschrijft de biodistributie en tumor-accumulatie van de nieuwe immunocytokine cergutuzumab amunaleukin (CEA-IL2v). Dit geneesmiddel bestaat uit een gemodificeerde variant van het cytokine interleukine-2 (IL2v) gekoppeld aan een antilichaam dat gericht is tegen carcino-embryonaal antigeen (CEA). Deze immunocytokine is ontwikkeld om doelgericht te werken bij tumorcellen die CEA tot expressie brengen, waar het een lokale immuunrespons kan veroorzaken door binding aan de interleukine-2 receptoren op lymfocyten. In deze studie onderzochten wij hoe dit middel zich over het lichaam verdeelt, en of het zich inderdaad ook naar de tumor begeeft. Hiertoe is CEA-IL2v gekoppeld aan een radioactief $^{89}$Zirconium ($^{89}$Zr) molecuul. Na toediening van $^{89}$Zr-CEA-IL2v kan het middel in de patiënt gevolgd worden door middel van $^{89}$Zr-positron emissie tomografie ($^{89}$Zr-PET). De resultaten van deze studie laten zien dat dit middel accumuleert in de lever, milt en lymfeknopen bij alle doses die gebruikt zijn. Accumulatie in de tumor werd echter pas gezien bij doses hoger dan 20 mg. Zoals verwacht op basis van de CEA-bindende eigenschappen, was tumor accumulatie het meest uitgesproken in tumoren die CEA tot expressie brengen. Een onverwacht resultaat van deze studie was dat de verdeling van dit middel veranderde na meerdere toedieningen. Na de vierde toediening zagen we dat de accumulatie in de lever hoger was dan bij de eerste toediening, terwijl de bloedspiegels, en de accumulatie in milt en tumor duidelijk minder werden. Onze hypothese is dat dit komt door ADAs, saturatie van interleukine-2 receptoren en/of bezetting van CEA bindingsplaatsen in de tumor. Deze resultaten bevestigen dat CEA-IL2v inderdaad doelgericht naar de tumor gaat, hoewel dit minder wordt gedurende de behandeling. Om hiervoor te compenseren zou de dosis gedurende de behandeling verhoogd moeten worden. Daarnaast laat deze studie zien dat het gebruik van een $^{89}$Zr-label geschikt is om de biodistributie van antilichamen te kunnen bestuderen, en illustreren de resultaten hoe belangrijk het is dat $^{89}$Zr-PET onderzoeken niet alleen na de eerste toediening plaatsvinden, maar ook tijdens de behandeling om veranderingen in de dispositie te kunnen waarnemen.

**Hoofdstuk 3.2** en **3.3** gaan over de eerste tekenen van effectiviteit van pembrolizumab, een antilichaam tegen het geprogrammeerde celdood (PD)-1 eiwit. Het PD-1 eiwit is een receptor die tot expressie komt op verschillende cellen van het immuunsysteem, zoals natural-killer cellen en cytotoxische ($CD^{8+}$) T-cellen. Activatie van PD-1 heeft een regulerend/remmend effect op het immuunsysteem. Als PD-1 geremd wordt, wordt deze immuunsuppressie opgeheven waardoor een versterkte immuunrespons optreedt. Beide hoofdstukken bevatten resultaten van dezelfde fase I studie waarin de effectiviteit en veiligheid van pembrolizumab werd onderzocht in 20 verschillende tumortypes. Dit proefschrift bevat resultaten van een aantal tumortypes. De volledige resultaten worden of zijn inmiddels separaat gepubliceerd. In **hoofdstuk 3.2** worden de resultaten van alle patiënten die behandeld zijn in het Nederlands Kanker Instituut-Antoni van Leeuwenhoek ziekenhuis gepresenteerd. Van de 17 patiënten met negen verschillende tumortypes, werd een antitumor effect van pembrolizumab waargenomen in tien patiënten. Deze patiënten hadden mesothelioom, nasofarynxcarcinoom, ovarium- of vulvacarcinoom of carcinoïde tumoren. In twee patiënten, één met nasofarynx- en één met ovariumcarcinoom, werden afnames van de tumor van 74% gezien en de behandeling werd goed verdragen. **Hoofdstuk 3.3** laat zien dat ook in patiënten met mesothelioom veelbelovende responsen hebben plaatsgevonden. Dit hoofdstuk bevat de resultaten van alle 25 patiënten die wereldwijd in dit cohort van de studie behandeld zijn. In deze groep werd bij 20% een afname van het tumorvolume >30% gemeten. De respons in mesothelioom duurde mediaan 12 maanden, en patiënten leefden mediaan 18 maanden. Dit is een relevante toename ten opzichte van de verwachte overleving van zes tot 11 maanden bij bestaande therapieën. Pembrolizumab bleek een acceptabel bijwerkingen profiel te hebben, die vergelijkbaar was tussen de verschillende tumortypes. De meest voorkomende bijwerkingen waren misselijkheid en moeheid, en dit heeft niet geleid tot onderbreking van de behandeling. Wel bestaat het risico op (ernstige) immuun-gemedieerde bijwerkingen, zoals hypothyreoïdie, huid- en oogtoxiciteit, als gevolg van de stimulerende werking op het immuunsysteem van pembrolizumab. Al met al geven de resultaten van deze studie aanleiding tot verder onderzoek naar de effectiviteit van pembrolizumab in grotere groepen van patiënten met mesothelioom, anus-, vulva-, kleincellig long-, nasofarynx- en cervixcarcinoom.

In **hoofdstuk 4** staat het gebruik van *BRAF* status als predictieve marker voor respons op anti-epidermale groei factor (anti-EGFR) monoclonale antilichamen in patiënten met CRC centraal. Anti-EGFR antilichamen zijn geregistreerd voor patiënten met gemetastaseerd CRC met EGFR expressie. Het is bekend dat mutaties in het KRAS eiwit leiden tot therapieresistentie omdat deze mutaties leiden tot activatie van de EGFR-signaleringsroute, zelfs in afwezigheid van het ligand EGF. Een vergelijkbaar resistentiemechanisme zou kunnen plaatsvinden bij patiënten met mutaties in het BRAF eiwit, omdat ook hier een EGFR-onafhankelijke activatie van de signaleringsroute optreedt. Verschillende studies tonen aan dat dit inderdaad leidt tot resistentie tegen therapie met anti-EGFR antilichamen, maar het bewijs is tot nu toe onvoldoende consistent bevonden om dit mechanisme te kunnen bevestigen. In deze literatuurstudie onderzochten wij welk bewijs er nu daadwerkelijk is voor het effect van *BRAF* mutaties op de werkzaamheid van anti-EGFR therapie. Wij vonden acht meta-analysen die consistent laten zien dat patiënten met *BRAF* gemuteerd CRC geen significant voordeel van anti-EGFR therapie hebben. De conclusie van dit hoofdstuk is dan ook dat deze patientgroep geen anti-EGFR therapie zou moeten krijgen, omdat de *BRAF* mutatie voorspellend is voor resistentie. In plaats daarvan zouden deze patiënten baat kunnen hebben bij chemotherapie of bij een combinatie van EGFR antilichamen en BRAF remmers. Deze combinatie wordt momenteel in klinisch onderzoek getest en laat, in tegenstelling tot monotherapie met EGFR antilichamen, veelbelovende resultaten zien.

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Emilie

**CURRICULUM VITAE**

Emilie van Brummelen was born on April 14th, 1989 in Rotterdam, The Netherlands. In 2007 she graduated from Gymnasium Beekvliet in Sint-Michielsgestel, after which she moved to Utrecht to study Pharmacy at Utrecht University. During this study, she performed a six-month research project at the department of Pharmaceutics at Utrecht University focusing on the development of polyomavirus derived virus-like particles via directed evolution. After this, she moved to Berlin for a three-month internship at Bayer Pharma, at the department of Regulatory Affairs. In 2012 she completed the Honours Programme of Pharmacy which included research projects about point-of-care glucose measurements in neonates at the Department of Clinical Chemistry of University Medical Centre Utrecht and the effects of broccoli on the CYP2D6 phenotype. After receiving her PharmD degree, she started her PhD research at the department of Clinical Pharmacology in the Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital in Amsterdam. Her research was conducted under supervision of prof. dr. Jan Schellens and prof. dr. Jos Beijnen, and focused on early phase clinical trials with targeted anticancer agents. The results of this research are presented in this thesis. In 2017, Emilie became a board certified clinical pharmacologist of the Dutch Society for Clinical Pharmacology & Biopharmacy.