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# Pharmacokinetics and Pharmacodynamics effects of Everolimus and Sorafenib combination: impact of doses and sequence of administration on the combination

Mévidette El Madani

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# Effets pharmacocinétique et pharmacodynamique de l'association d'everolimus et de sorafénib : L'impact des doses et des schémas d'administration sur la combinaison

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## List of Abbreviations

Abb.	Full term
<b>[(18F) FLT)-PET</b>	<b>[18F]-fluorodeoxy-L-thymine</b>
<b>(AUC<sub>T</sub>)</b>	<b>Areas under the concentration versus time curves within the dosing interval</b>
<b>4E-BP1</b>	<b>eIF4E-binding proteins</b>
<b>AEs</b>	<b>Adverse event</b>
<b>AGT</b>	<b>Human <i>O</i><sup>6</sup>-alkylguanine-DNA alkyltransferase</b>
<b>AKT Total</b>	<b>Protein kinase B</b>
<b>AKT1</b>	<b>Serine/Threonine Kinase 1</b>
<b>AUC</b>	<b>Area under the curve</b>
<b>CI</b>	<b>Confidence Interval</b>
<b>Cl/F</b>	<b>Apparent oral clearance</b>
<b>C<sub>max</sub></b>	<b>Peak concentration</b>
<b>CR</b>	<b>Complete response</b>
<b>CRF</b>	<b>Case report form</b>
<b>CTCAE</b>	<b>Common terminology criteria of adverse events</b>
<b>CV</b>	<b>Coefficient of variation</b>
<b>CYP3A4</b>	<b>Cytochrome P450 3A4</b>
<b>DCE-MRI</b>	<b>Dynamic contrast enhanced magnetic resonance imaging</b>
<b>DLT</b>	<b>Dose limiting toxicity</b>
<b>EGFR</b>	<b>Epidermal growth factor receptor</b>
<b>EMA</b>	<b>European medical agency</b>
<b>ERK Total</b>	<b>Total extracellular signal regulated kinase</b>

<b>FOCEI</b>	<b>First order conditional estimation method with an interaction option</b>
<b>GEMM</b>	<b>Genetically engineered mouse model</b>
<b>HCC</b>	<b>Hepatocellular Carcinoma</b>
<b>HER2</b>	<b>Human epidermal growth factor receptor2</b>
<b>IBW</b>	<b>Ideal Body Weight</b>
<b>IC50</b>	<b>Inhibitory concentration 50</b>
<b>IOV</b>	<b>Inter-occasion variability</b>
<b>Ka</b>	<b>Absorption rate constant</b>
<b>Abb.</b>	<b>Full term</b>
<b>KRAS</b>	<b>V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog</b>
<b>LC-MS/MS</b>	<b>Liquid chromatography MS/MS</b>
<b>M&amp;S</b>	<b>Modeling and simulation</b>
<b>MBDD</b>	<b>Model based drug design</b>
<b>MEK</b>	<b>Mitogen activated protein kinase</b>
<b>MH</b>	<b>Morris Hepatoma</b>
<b>MP</b>	<b>Molecular profiling</b>
<b>MTA</b>	<b>Microtubule-targeting agents</b>
<b>MTD</b>	<b>Maximum tolerated dose</b>
<b>mTOR</b>	<b>Mammalian target of rapamycin</b>
<b>NONMEM</b>	<b>Non-linear mixed effect modeling</b>
<b>NSCLC</b>	<b>Non small cell lung cancer</b>
<b>OBD</b>	<b>Optimal biological dose</b>
<b>p70-S6K</b>	<b>Phosphorylated p70 ribosomal protein S6 kinase</b>
<b>pAKT</b>	<b>Phosphorylated protein kinase B</b>
<b>PBMCs</b>	<b>Peripheral Blood Mononuclear cells</b>



<b>PD</b>	<b>Pharmacodynamics</b>
<b>PDXs</b>	<b>Patient derived xenografts</b>
<b>p-ERK</b>	<b>Phosphorylated extracellular signal regulated kinase</b>
<b>PFS</b>	<b>Progression free survival</b>
<b>PGDE</b>	<b>Pharmacologically guided dose escalation</b>
<b>PgP</b>	<b>Phosphorylated Glycoprotein P</b>
<b>PI3K</b>	<b>Phosphatidylinositol 3-kinase</b>
<b>PIK3CA</b>	<b>Phosphatidylinositol-4,5-Bisphosphate3-Kinase Catalytic Subunit Alpha</b>
<b>PK</b>	<b>Pharmacokinetics</b>
<b>PK-PD</b>	<b>Pharmacokinetics-pharmacodynamics</b>
<b>PR</b>	<b>Partial response</b>
<b>PtdInsP3</b>	<b>Phosphatidylinositol 3,4,5-trisphosphate</b>
<b>PTEN</b>	<b>Phosphatase and tensin homolog</b>

<b>Abb.</b>	<b>Full term</b>
<b>Q/F</b>	<b>Intercompartmental clearance</b>
<b>R&amp;D</b>	<b>Research and development</b>
<b>RAF</b>	<b>Serine/threonine specific protein kinases</b>
<b>RAS</b>	<b>Reticular activating system</b>
<b>RP2D</b>	<b>Recommended phase 2 dose</b>
<b>RSE</b>	<b>Relative standard error</b>
<b>RTK</b>	<b>Receptor tyrosine kinase</b>
<b>SAEs</b>	<b>Serious adverse event</b>
<b>SIGMA</b>	<b>Exponential residual error</b>
<b>SOC</b>	<b>System Organ Class</b>

<b>SUSARs</b>	<b>Suspected unexpected serious adverse reactions</b>
<b>TRAIL</b>	<b>Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand</b>
<b>UGT1A1</b>	<b>UDP-glucuronosyltransferases 1A1</b>
<b>UGT1A9</b>	<b>UDP-glucuronosyltransferases 1A9</b>
<b>V<sub>2/F</sub></b>	<b>Peripheral volume of distribution</b>
<b>V<sub>dcentral</sub></b>	<b>Central volume of distribution</b>
<b>VEGF</b>	<b>Vascular endothelial growth factor</b>
<b>VEGFR1</b>	<b>Vascular endothelial growth factor receptor 1</b>
<b>VEGFR2</b>	<b>Vascular endothelial growth factor receptor 2</b>
<b>VPC</b>	<b>Visual predictive check</b>

## Introduction

The translation of cancer research to successful clinical application has been proved to be very challenging over the past decade. The attrition rate of drug development remains high despite the efforts and the financial investments that have been brought by many different parties including scientists, researchers and pharmaceutical companies.

Only 5% of agents that have anticancer activity in preclinical development are licensed after demonstrating sufficient efficacy in phase III testing, which is much lower than, other diseases. This issue involved also many new cancer agents including microtubule-targeting agents (MTA) that were withdrawn or suspended with 40–50% of development programs being discontinued even in clinical Phase III <sup>1,2</sup>.

Diverse reasons were reported as factors contributing for the high attrition rate of anticancer agents<sup>3</sup>. The concepts used for development of cytotoxic drugs were not adequate for new targeted agents: toxicity based escalation trials, MTD. Therefore, limitations and major challenges for the research based drug development could be summarized in the following:

- *Poorly predictive preclinical models in cancer research*: the limitations of preclinical tools such as inadequate cancer-cell-line and mouse models might explain the challenging mission of the scientists to make a discovery that will have an impact in the clinic <sup>4</sup>. Despite the progress of genetically engineered mouse model (GEMMs) and patient derived xenografts (PDXs), these models still not widely implemented <sup>5</sup>. However, recently, GEMMs have been used to identify the importance of mTOR and EGFR inhibitors in neuroendocrine cancers, leading to

the successful translation of mTOR inhibitors into clinical practice in this tumor type <sup>6,7</sup>. PDXs are also increasingly used to guide personalised therapy <sup>8,9</sup>.

- *Lack of reliability of published data*: An analysis by Prinz F et al., 2011 was based on input received from 23 scientists and collected data from 67 projects, revealed that in almost two-thirds of the projects there were inconsistencies between published data and in-house data. This concern has been addressed based on what some scientists have claimed about the presentation of specific experiments that supported their underlying hypothesis which were not reflective of the entire data set. Also, data were not routinely analyzed by investigators blinded to the experimental versus the control group. On the other hand, in studies for which findings could be reproduced, authors had paid close attention to controls, reagents, investigator bias and describing the complete data set <sup>10</sup>.
- *Starting dose determination*: endpoints based on optimal biological doses (OBD) were used to determine the recommended phase 2 dose (RP2D) for several FDA approved agents, such as bevacizumab, imatinib and vismodegib, as they didn't reach an established MTD in the phase I trial. Accordingly, a new approach was set using PK or PD as an endpoint to determine biologically active dose in preclinical experiments. This could be applied alongside with preclinical toxicology data to inform starting dose decisions. This binomial approach has the potential to reduce the number of dose escalations while keeping an optimised benefit /risk ratio. A number of conditional

and accelerated approvals have been granted based on phase II data relying on patient benefit <sup>11 12</sup>.

- *Patient selection*: Multiple genomic aberrations that drive oncogenesis may act as treatment targets. Therefore, the identification of a sufficient number of patients with a specific molecular aberration can significantly slow clinical trial accrual as the majority of these abnormalities have been reported with low frequency. In these cases multi-center studies with frequent communications between investigator sites should ameliorate these limitations. Geographic heterogeneity due to spatial variations in molecular aberrations has been demonstrated within a single tumor, or between different lesions. Multiple tumor biopsies, ultra deep sequencing and non-invasive tumor imaging could potentially overcome the limitations of geographic heterogeneity <sup>13-15</sup>.
- *The concept of target-based drug discovery with the related complexity of target selection*: the reliance on standard criteria for evaluating tumour response and the challenges of selecting patients prospectively also play a significant part in the success rate of a new molecule to be translated to clinic <sup>16</sup>. The disappointing results in the clinic produced by some anticancer agents like mitotic kinases could be partially related to the lack of a balanced benefit /risk ratio as their efficacy was at the expense of high toxic effects. This might be explained by a non 'druggable' tumor cells which means that the activity of the key target of the anticancer agents was not inhibited in the tumor cells<sup>17</sup>.
- *Complexity of clinical trial, together with increasing demands from regulatory authorities and payers* <sup>18</sup>: Despite the superior efficacy of combination treatment over single agent drugs as demonstrated by

numerous studies, there are few examples where single drugs are approved in a combination, but not as single agent. Such trials are more complex and typically require an active control comparator. In addition, most of drugs are initially approved in advanced disease, and in later lines of treatment when cancer is biologically much more difficult to treat <sup>19</sup>. This trend underlines the need for larger studies, longer time to endpoints, and the requirement that a new drug be superior or not inferior to existing drugs. Strategies to reduce the chance of overlooking a valuable drug might include novel study designs, better predictive models, and perhaps changes in regulatory approach <sup>20</sup>.

All the challenges listed above makes it necessary for pharmaceutical companies to reconstruct their research and development (R&D) concepts to overcome the reduced R&D efficiency. These companies need to identify the right growth strategies, need to build up the right core competences for drug R&D internally, and to put pragmatic solutions to ensure a sustainable investment in R&D to generate a steady flow of new innovative drugs. This could be through the implementation of open innovation processes, hire people who are open-minded, able to work with different cultures, form more strategic alliances to better utilize external partnerships <sup>21</sup>.

As a consequence, new drug development strategies meant to identify the best doses and dosing schedules of novel targeted agents have been proposed, among them, determination of the OBD appears promising. Indeed, identification of the minimal dose associated with optimal biological effect through measurement of target inhibition and

pharmacokinetics (PK) analysis might be a good alternative for defining the recommended phase II trial dose. However, although OBD is an attractive endpoint for defining the RP2D of novel targeted agents, there is no data about the actual relevance in terms of clinical efficacy of this endpoint.

An example of the importance of setting a non-traditional endpoint in oncology trials was investigated in a literature review analysing phase I studies involving 31 single agents used in treatment of cancer. This review had for objective to describe methods of dose selection, including recommended phase II dose; and to characterize the contribution of correlative studies to dose selection. It was demonstrated that the primary basis for the dose recommendation was toxicity. Meanwhile, pharmacokinetic data were the primary basis for the final dose selection in 11 of the 52 studies. supposing that there is strong preclinical evidence demonstrating an association between drug levels and target inhibition. Other less commonly cited reasons included measures of molecular drug effects in tumor or surrogate tissue or functional imaging studies.

Furthermore, tumor correlative studies were the primary basis for dose selection in only one trial that evaluated an EGFR antibody given to patients with non–small-cell lung cancer or head and neck cancer Correlative studies to evaluate molecular measures (e.g., assessment of change in mRNA before and after therapy) of drug effect using surrogate tissues such as peripheral blood mononuclear cells (PBMCs), skin, and buccal mucosa were more commonly incorporated into phase I trials than were studies of tumor tissue <sup>22</sup>.

### **Dose escalation methods in early phase trial design**

The recommended dose for phase 2 trials is selected based on prespecified dose levels of therapeutic doses data established in phase I trials. depending on which one best fits the definition of acceptable toxicity set a priori. These early phases I trials are designed following specific guiding principle for dose escalation methods to treat as many patients as possible within the therapeutic dose range. taken into consideration patient's safety and rapid accrual to the study. Dose escalation methods for phase I cancer clinical trials fall into two broad classes: the rule-based designs, which include the traditional 3+3 design and its variations, and the model-based designs. All of these methods were developed assuming that both efficacy and toxicity increase proportionally with dose. Consequently, these methods have used toxicity as the primary endpoint. In contrast to molecularly targeted agents, efficacy may occur at doses that do not induce clinically significant toxicity. Drug-related biological effects has been suggested as an alternate primary endpoint besides toxicity<sup>23-26</sup>.

### **Rule-Based Designs**

#### ***Traditional 3+3 Design***

The traditional 3+3 design is the first rule-based design to be used widely in clinical practice because it is safe and simple to implement. It was claimed that the traditional 3+3 design is the safest in terms of grade 3 or 4 nonhematologic and grade 4 hematologic toxicities<sup>27</sup> Another review found an increased response rate but no increased risk of toxicity when inpatient dose escalation was allowed<sup>28</sup>. The traditional rulebased method has been successful in establishing safe recommended, doses for phase II trials for



anticancer agents, either cytotoxic or targeted agents approved by FDA and used worldwide in clinical practice<sup>66</sup>.

The general principle of this design is to escalate or de-escalate the dose with diminishing fractions of the preceding dose depending on the absence or presence of severe toxicity in the previous cohort of treated patients. In addition, the accrual of three patients per dose level provides additional information about pharmacokinetic interpatient variability. However, a disadvantage of this design is that it involves an excessive number of escalation steps, which results in a large proportion of patients who are treated at subtherapeutic dose<sup>29</sup>.

### ***Accelerated Titration Designs***

Accelerated titration designs are classified as rule-based designs, because the patient assignment to doses is based on prespecified rules, although features of model-based design are implemented. The advantage of accelerated titration design over 3+3 dose escalation, is inpatient dose escalation giving the chance to some patients to be treated at higher and at the same time most effective doses in smaller time frame<sup>29</sup>. In addition, data from all patients, cumulative toxicity, and interpatient variability can be fit to a model to establish the RP2D. While the drawbacks of such method is the difficulty of interpretation of the results when inpatient dose escalation is allowed and consequently uncertainty about the RP2D<sup>30</sup>.

### ***Pharmacologically Guided Dose Escalation (PGDE)***

The PGDE method is another variation of the traditional 3+3 design. This approach assumes that dose-limiting toxicities can be predicted by

plasma drug concentrations and that animal models can accurately reflect this relationship in humans<sup>31</sup>. The PGDE method has two stages: stage 1 prespecified plasma exposure defined as Area under the curve of drug concentration (AUC) extrapolated from preclinical data. Stage 2: pharmacokinetic data obtained for each patient in real time to determine the subsequent dose level. This method has not been widely used in clinical practice due to practical obstacles, including: 1) logistic difficulties in obtaining realtime pharmacokinetic results. 2) problems in extrapolating preclinical pharmacokinetic data to phase I studies with different treatment schedules; 3) risk of exposing the next patient to a highly toxic dose due to interpatient variability in drug metabolism<sup>31</sup>.

### ***Dose escalation models of combination phase I trials***

The combination of two or more agents in the clinic and the choice of dose levels of each of the combined drugs should be based on a strong scientific rationale such as preclinical data and/or the current standard treatments in tumor types. Existing preclinical models often focus on the antitumor effects of drug combinations rather than their potential for creating severe toxicities. Consequently, when rule-based design is used for phase I trials, the dose of each drug should be carefully chosen to reach maximum tolerated dose (MTD). Uncertainty about the optimal drug combination that yields the best therapeutic index may be overcome by the development of bayesian model based designs specific for combination trials<sup>32-36</sup>.

## **Designs for Trials of Molecularly Targeted Agents (MTAs)**

The setting of an endpoint for clinical trials designs of molecularly targeted agents based only on measurement of target inhibition has been proven to be suboptimal. Pharmacokinetics endpoints such as plasma drug concentration associated with biological activity in preclinical studies should also be considered when selecting a recommended dose for phase II trials for these agents. In a limited number of reported clinical trials of molecularly targeted agents, specific designs were developed to define the recommended dose for phase II trials.

For example, Friedman et al.,1998 introduced the concept of a biological endpoint of Human *O*<sup>6</sup>-alkylguanine-DNA alkyltransferase (AGT) inhibition in a dose escalation phase I trial of patients undergoing craniotomy for malignant glioma. Other proposals for phase I trial designs specific for molecularly targeted agents Mandrekar et al.,2007 developed a bayesian-based method that incorporates toxicity and a biological endpoint for molecularly targeted agent combinations <sup>37-40</sup>.

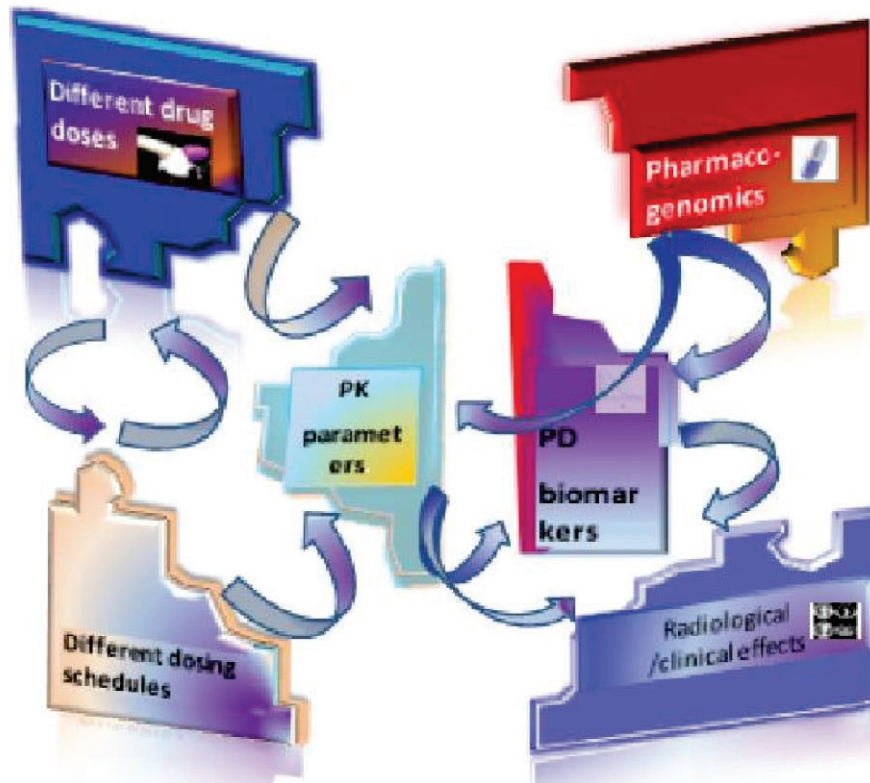
## **Model based drug design (MBDD)**

The ability to identify critical targets involved in cancer cell growth and survival is one of the reasons why oncology drug development is witnessing a significant shift from classical chemotherapy regimens to selective and potent targeted molecular therapeutics. Unlike classical cytotoxic chemotherapy drugs, these modern agents target specific proteins that are involved in tumor growth processes. Despite the uncontestable scientific improvement offered by their emergence, the development of

these targeted drugs brings new issues. One of the greatest challenges to be addressed is the optimal development of targeted drug combinations.

Indeed most of these novel selective drugs are not sufficiently effective in avoiding a relapse when used as antitumor single agents, thereby warranting their development in combinations <sup>41,42</sup>. Designing proper trials for evaluation of the best doses and dosing schedules of targeted agent associations and acknowledging potential pharmacokinetic (PK) and pharmacodynamic (PD) interactions, has recently been recognized as a major challenge for the next century <sup>43</sup>. Mathematical models able to describe biological phenomena at different complexity scales by using equations and to simulate the effects induced by changes in experimental conditions, may provide some solutions to this issue <sup>44-46</sup>. Indeed, based on the data of adequately designed clinical trials, mathematical modeling can define the best dose and dosing schedule of drugs using simulations, as demonstrated during the development of everolimus <sup>47</sup>.

We assume this tool may be used to identify the optimal doses and dosing schedules of combined study agents based on maximization of the simulated expected benefit/toxicity ratio. However, such a strategy requires the data from adequately designed early-phase trials, called multiparameter Phase I trials, where different doses and dosing schedules are investigated together with multiple biological, radiological and clinical parameters. These data are required so the links between doses, dosing schedules, PK, PD, pharmacogenetics, radiological and clinical effects can be quantified by the models (**Figure 1**).



**Figure (1):** Relationships between all parameters (doses, dosing schedules, pharmacokinetic, pharmacogenomic, pharmacodynamic and clinical effects) required to optimize study drug administrations. PD: Pharmacodynamic; PK: Pharmacokinetic

### *Challenges to model based drug design (MBDD)*

The first challenge is the natural resistance to change in a highly conservative industry such as biopharmaceuticals. This was argued by the unopeness of regulators to approve deviations from traditional approaches and methods previously used. Although, this mindset have changed and regulatory authorities have themselves played a role in the modernisation of drug developement, the regulatory position towards MBDD is still highly dependent on the division and disease under consideration <sup>48</sup>. Another challenge is the failure to meet the expected

benefit of MBDD leading to frustration and decreased confidence in MBDD. It is crucial that those leading the MBDD implementation efforts to provide a realistic picture of the advantages of modeling and simulation (M&S) in order to maintain the credibility of the possible application of modeling technique into practice <sup>49</sup>.

### *Opportunities to model based drug design (MBDD)*

Introducing the notion of new discipline such as systems pharmacology that lies at the interface between systems biology and PK/PD in order to select compounds that are most likely to translate to clinical efficacy and safety through iterative learning from modelling and experimentation <sup>50</sup>. Also clinical studies based on optimal sampling techniques provide more accurate and precise estimates of model parameters, resulting in better predictions of clinical outcomes <sup>51</sup>. If acceptable utility index M&S can be used to determine optimal treatments (e.g. dosage regimen), which can subsequently lead to effective study designs aimed at identifying or confirming such optimal treatments<sup>52</sup>. Bayesian methods, which combine previous and current information, are particularly useful in this context <sup>53</sup>.

### **Combination drug development**

Molecularly targeted agents can be combined to inhibit multiple components within a signaling pathway to evade resistance mechanisms or to target a distinct oncogenic processes. Combination strategies may include either additive or synergistic drug combinations of the same mechanism or the addition of a second agent with a different mechanistic activity to reverse resistance mechanisms. Between January 2006 and June 2016, about nine molecular targeted agents (MTAs) combinations were

approved by the US Food and Drug Administration (FDA) for use in adult solid malignancies, compared with approximately 40 approved single-agent MTAs and approximately 20 MTA–chemotherapy combinations <sup>54</sup>.

These combination approvals are based on randomized phase III or phase II trial data demonstrating improved progression-free survival or overall survival compared with the established standard of care. Notably, in these nine approved combinations, MTAs are used at their single-agent recommended dose, without substantial increase in toxicity. Additionally, in seven out of the nine combinations—with the exceptions of lenvatinib and everolimus, and nivolumab and ipilimumab—established predictive biomarkers are utilized for molecularly based patient selection <sup>55-63</sup>.

Predictive biomarkers are protein or genome markers, correlate with the success of specific therapies and thus help select the optimal therapies for patient care. For example, ER and PgR status predict response to endocrine therapy <sup>64</sup>, and Human epidermal growth factor receptor2 (HER2) amplification predicts response to HER2-targeted therapies such as trastuzumab <sup>65</sup>.

Adaptive Bayesian model-based designs may be a good statistical option to be considered for the complex variables associated with combination MTAs, by incorporating pre-study probability of toxicity and updating such probability with real-time adverse event (AEs) data to inform dose-escalation decisions <sup>32,33,35</sup>. In simulation studies, adaptive designs were found to maximize the number of patients treated at or near the MTD compared with rule-based designs. Adaptive designs allow prospective modifications on aspects of the trial as the data evolve, offering greater flexibility to researchers <sup>66,67</sup>.

Currently, there is no preferred dose-escalation design for combination MTAs. The choice of the most appropriate dose schedule selection and dose-finding method should be informed by knowledge of the nonclinical and clinical pharmacology of the agents of interest and based on consultation between experienced clinical researchers, sponsors, and statisticians. Comprehensive pharmacokinetic evaluation and pharmacodynamic assessment of tumors in early phase trials are vital to assess for target modulation and to mechanistically characterize on- and off-target toxicities <sup>34,68,69</sup>.

### **The role of personalised medicine in patient selection**

Despite the exciting potential of personalized medicine, currently there are only a few selected diseases and molecular subtypes in cancer for which there are therapy approaches with proven efficacy. Examples of this include anti-HER2-targeted therapy for *HER2*-amplified breast cancer, EGFR-targeted therapy for *EGFR*-mutant lung tumours, and the mutationselective Serine/threonine specific protein kinases (RAF) and Mitogen activated protein kinase inhibitors (MEK) for *BRAF*-mutant melanoma <sup>70-73</sup>.

Initial researches have not only revealed the immense complexity of the cancer genome but also the striking inter-, and most notably, the intratumour heterogeneity at the whole-genome level in solid tumours<sup>74</sup> be correlated to organ-specific metastasis <sup>75,76</sup>. This remarkable tumour heterogeneity represents a major challenge to personalized medicine and biomarker development <sup>77</sup> and could probably in part explain the mixed responses to targeted therapies <sup>78</sup>.



But despite some limitations, personalized treatment has provided us with some fruitful examples in solid tumours up to the point of changing the natural evolution of two of the most fatal tumour types (Non small cell lung cancer "NSCLC" and melanoma), for which standard research had proven quite unsuccessful. The first of these drugs, crizotinib (Xalkori™, Pfizer), was tested in a phase I–II clinical trial in NSCLC patients having chromosomal translocation resulting in the production of a novel type of fusion protein, EML4–ALK, with constitutive activation of the kinase activity of the *ALK* oncogene. The results appeared quite spectacular, with 57% of partial responses (PR) with one confirmed complete response (CR). In this preliminary trial, the progression free survival (PFS) at 6 months already reached 72%<sup>79,80</sup>.

The second example is a large multicentric phase III trial comparing vemurafenib with dacarbazine in 550 patients with advanced melanoma expressing the *B-RAF* V600E mutation showed the clear superiority of vemurafenib over dacarbazine, with a median PFS of 5.3 months in the vemurafenib arm versus 1.6 months in the dacarbazine one. This positive effect had a significant benefit on overall survival<sup>81</sup>.

In addition, genomic-based trials can also generate valuable information regarding cancer biology; clinically qualify potential predictive biomarkers; accelerate patient benefit; and assist in the decision on whether a novel targeted agent warrants further development<sup>82</sup>. Some studies have shown that real-time molecular profiling (MP) of tumours from actual patients and treatment with matched targeted agents can increase response rates and improve the time to progression compared with unselected therapies<sup>83</sup>.

## I-Hypothesis

- Combination of sorafenib and everolimus is a promising regimen for treatment of patients with advanced solid tumors.
- Doses and dosing schedules of sorafenib may impact on everolimus PK parameters, on everolimus tumor exposure and thus on pharmacodynamic effects, along with on toxicity induced by the combination regimen
- The benefit-toxicity ratio of the combination may be improved by optimizing administration sequence and doses of each drug using PKPD modeling studies. Simulations might enable identification of the respective doses and dosing schedules able to maximize efficacy and minimize toxicity.
- A phase 1 trial in which dosing schedules and doses of study drugs vary may help better understand the relationships between dosing regimens, drug doses, PK and PD effects, and generate useful data for modeling and simulation works.

## II-Rationale

### • **PI3K-AKT-mTOR pathway in cancer drug discovery**

The Phosphatidylinositol 3-kinase (PI3K) pathway is activated in cancer, making this an optimal target for therapy. Some drugs either in clinical use or preclinical evaluation originally developed for other purposes have been demonstrated to directly or indirectly target PI3K signalling. These include mammalian target of rapamycin (mTOR) inhibitors of the ‘rapalog’ family of rapamycin analogues, ether lipids (such as perifosine

and miltefosine), and inhibitors of epidermal growth factor receptor (EGFR), HER2/*neu*, c-Kit, platelet-derived growth factor receptor (PDGFR) and BCR–ABL<sup>84</sup>. However, except for mTOR inhibitors, which seem to solely target the PI3K pathway, it is still not very clear whether functional outcomes of these drugs relate to inhibition of the PI3K pathway or to other effects. As the PI3K pathway is a crucial regulator of survival during cellular stress, and given that tumours frequently exist in intrinsically stressful environments with limited nutrient and oxygen supply and low PH, PI3K pathway inhibitors is likely to find optimal efficacy in combination with other signal transduction inhibitors and with chemotherapy or radiation therapy<sup>85</sup>.

- **Overview for the P3K-AKT-mTOR pathway**

The mTOR pathway is a key regulator of cell growth and proliferation and increasing evidence suggests that its deregulation is associated with human diseases, including cancer and diabetes. The mTOR pathway integrates signals from nutrients, energy status and growth factors to regulate many processes, including autophagy, ribosome biogenesis and metabolism. Phosphatidylinositol 3-kinase (PI3K) phosphorylates phosphatidylinositol biphosphate (PIP2) on the 3-OH position to produce Phosphatidylinositol 3,4,5-trisphosphate (PtdInsP3) (**Figure 2**)<sup>86-88</sup>.

87,89. 90.

PI3K signaling is activated in human cancers via several different mechanisms. Increased PI3K signaling is often due to direct mutational activation or amplification of genes encoding key components of the PI3K pathway such as Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic

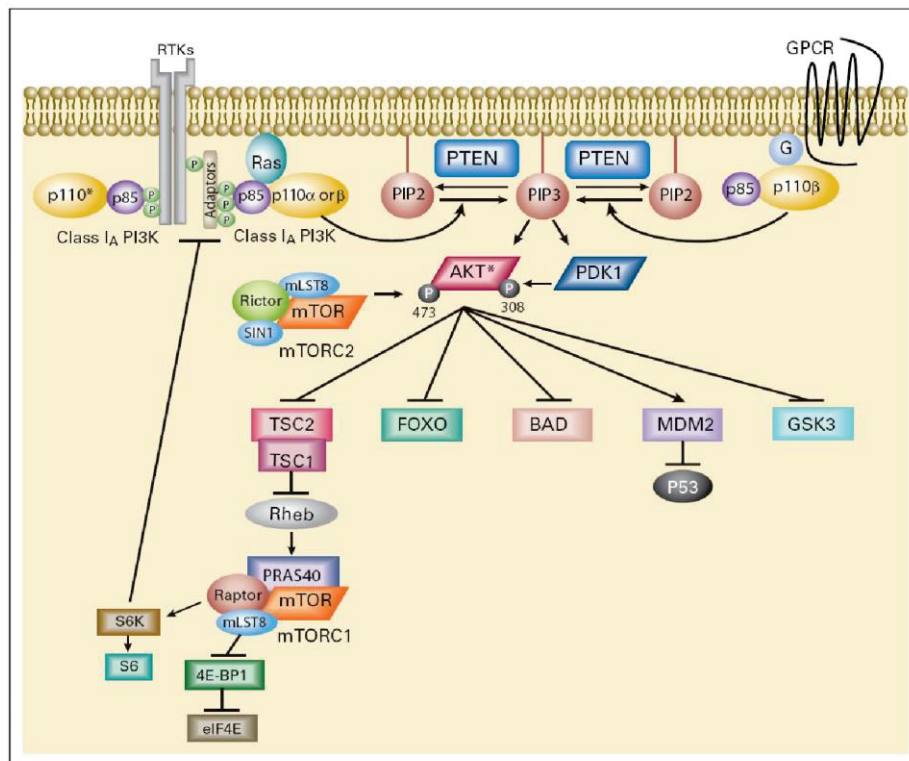
Subunit Alpha (PIK3CA) and Serine/Threonine Kinase 1 (AKT1), or loss of PTEN. Genetic alterations in several components of the PI3K signaling pathway have been reported. PI3K also can be activated by genetic mutation and/or amplification of upstream receptor tyrosine kinases (RTKs), and possibly by mutationally activated RAS<sup>91</sup>.

The most common genetic alteration of the PI3K signaling pathway found in human cancer is inactivation of the PTEN tumor suppressor gene. Inactivation of PTEN leads to loss of its lipid phosphatase activity, causing accumulation of PIP3. The majority of somatic mutations in PTEN leads to protein truncation.<sup>92,93</sup>

In normal epithelial cells, PI3K is often activated downstream of RTK signaling. In cancers, these RTKs are often mutated, amplified, or overexpressed, causing aberrant PI3K activation. When therapies targeting RTKs are effective, they invariably lead to loss of PI3K signaling. For example, PI3K is activated by epithelial growth factor receptor (EGFR) in lung cancers harboring somatic activating mutations in EGFR, and by human epidermal growth factor receptor 2 (HER2) in breast cancers with HER2 amplification. In these cancers, EGFR or HER2 phosphorylates the kinase-dead ErbB3 that, in turn, directly binds and activates PI3K. Thus, when these cancers are successfully treated with EGFR- and HER2targeted therapies, respectively, PI3K signaling is turned off and the cells undergo cell death<sup>94,95</sup>. The small GTPase reticular activating system (RAS) is also frequently mutated in human cancers, and PI3K is an effector of RAS-mediated oncogenic signaling.<sup>96 97-99</sup>.

Although PI3K activation may be necessary for K-RAS-induced tumorigenesis, preliminary studies suggest that inhibition of PI3K signaling

alone may not be sufficient to shrink established tumors *in vivo* or effectively treat K-RAS–mutated cancer cell lines *in vitro*. These findings underscore the difference between killing established cancers and blocking tumorigenesis and cell transformation. Furthermore, these studies suggest that established cancers with V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations may not be sensitive to single-agent PI3K pathway inhibitors<sup>100,101</sup>.

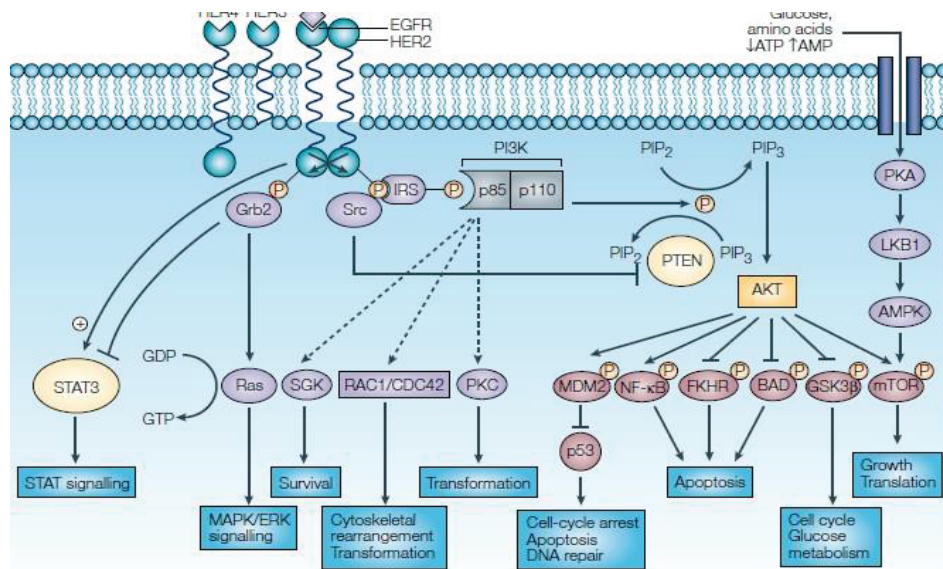


**Figure (2):** The phosphatidylinositol 3-kinase (PI3K) signaling cascade. PI3K signaling impacts on cell growth, survival, and metabolism. Arrows represent activation, while bars reflect inhibition. A negative feedback loop has been described from the downstream target S6 kinase (S6K) to the adaptor protein IRS-1. RTK, receptor tyrosine kinase; GPCR, G-protein coupled receptor; P, phosphate; G, G protein; PTEN, phosphatase and tensin homolog; IRS-1, insulin receptor substrate 1; eIF4E, eukaryotic initiation factor 4E; S6, ribosomal S6 protein; PIP2, phosphatidylinositol 4,5-bisphosphate; mTORC2, rapamycin (mTOR)–containing protein complex 2. (\*) p110 alpha, beta, or delta<sup>102</sup>.

- **Overview of the RAS-RAF-ERK pathway**

The RAS serine/threonine kinase isoforms (A-RAF, B-RAF and RAF1 or C-RAF) are the first kinases in the RAS-RAF-ERK cascade and are pivotal regulators of cellular proliferation and survival (**Figure 3**). Dysregulated activation of RAF pathway, which can be independent of RAF kinase activity, might be implicated in tumorigenesis and the progression of several solid tumour types. Dysregulated signaling pathway activation through RAF kinase isoforms is detected in ~30% of human cancers. Wild-type RAF1 is frequently hyperactivated in a wide range of human solid tumours because of constitutively active upstream oncogenic RAS mutants, or the overexpression of upstream growth factors and/or their RTKs in the absence of oncogenic mutations. Furthermore, constitutively active RAS oncogenes (particularly *K-RAS*) are common in human solid tumours, including pancreatic and colorectal cancers most commonly and to a lesser extent kidney tumour.<sup>103</sup>

Collaboration between different pharmaceutical companies provided further validation of RAF1 as a target for anticancer drug design. They demonstrated that mice with colon, pancreatic or fibrosarcoma human tumour xenografts harbouring oncogenic *k-ras*, and expressing an MEK<sup>104-</sup>  
106.



**Figure (3):** Description of the RAS-RAF-ERK and PI3K-AKT-mTor signaling pathways.<sup>107</sup>

### • Overview of VEGF and VEGFR

Angiogenesis, the formation of new blood vessels from pre-existing ones, plays a central role in the process of tumor growth and metastasis. The proliferation of endothelium and formation of new blood vessels further the size of solid tumors. It is expected that blocking angiogenesis will be an efficient therapeutic approach against many tumor types<sup>108</sup>. The key signaling system that regulates proliferation and migration of endothelial cells are vascular endothelium growth factor (VEGF) and their receptors (VEGFR-1, 2 and -3). VEGFR-2, a receptor with higher affinity and greater kinase activity, is more important in the direct regulation of angiogenesis, mitogenic signaling, and permeability-enhancing effects<sup>109</sup>. VEGFRs are expressed at high levels in many types of human solid tumors, including glioma, lung, breast, renal, ovarian and gastrointestinal tract carcinomas. Inhibition of VEGFR has emerged as a potential therapy method for cancers and it has been clinically validated with FDA-approvals of bevacizumab, sorafenib, and sunitinib 110-115.

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### III. Drugs acting on cancer signaling pathways RAS-RAF/ERK and P3K-AKT-mTOR

#### a. Sorafenib

##### ✦ *Targets for Sorafenib*

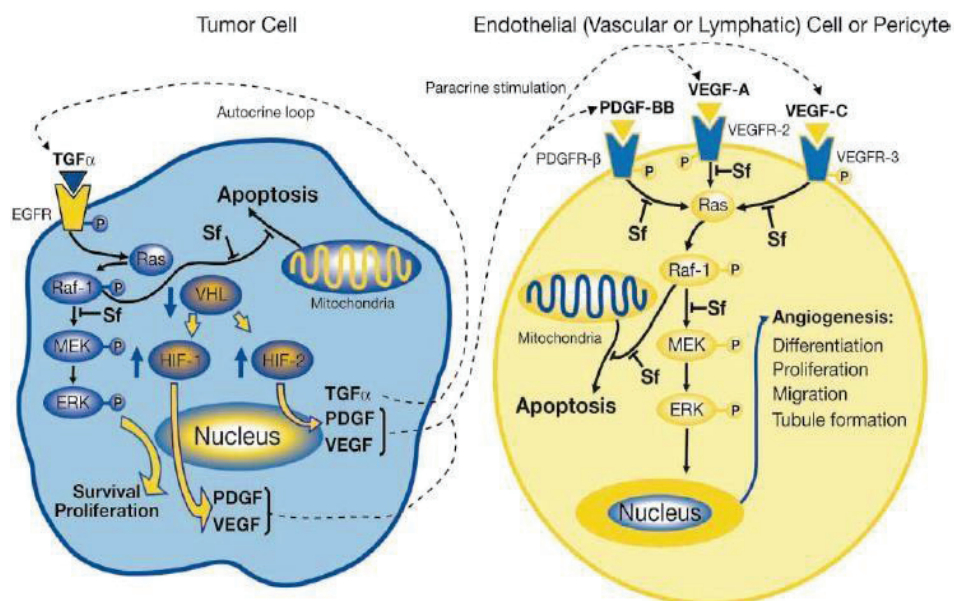
Sorafenib has multiple known protein kinase targets (**Figure. 4**) as identified in biochemical and cellular assays in vitro <sup>104,116</sup>. In an initial screening, sorafenib was identified as a potent inhibitor of Raf serine/threonine kinase isoforms in vitro. Sorafenib has since been shown to have potent inhibitory effects on other Raf isoforms in biochemical assays, with an order of potency of Raf-1 > wild-type B-Raf > oncogenic B-Raf V600E. However, sorafenib does not inhibit MEK-1 or extracellular signal-regulated kinase (ERK)-1 kinase activity in vitro. Sorafenib has been shown to inhibit ERK signaling, as measured by the reduction in ERK phosphorylation, in several cell lines from both hematopoietic malignancies and solid tumors. Sorafenib is capable of inhibiting ERK signaling in tumor cell lines with wild-type K-Ras and BRAF and no known oncogenic activation of the ERK pathway as well as in cell lines containing oncogenic K-Ras or B-Raf. <sup>104,116</sup>.

The antiproliferative activity of sorafenib varies widely depending on the oncogenic signaling pathways driving proliferation. In addition to targeting Raf serine/threonine kinases, sorafenib also potently inhibits the proangiogenic vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, VEGFR-3, and platelet-derived growth factor receptor (PDGFR-h) tyrosine kinases in biochemical assays in vitro. In cellular assays, sorafenib inhibits the VEGF-mediated autophosphorylation



of VEGFR-2 (human endothelial cells and NIH 3T3 fibroblasts expressing VEGFR-2), VEGFR-3, and PDGF-mediated autophosphorylation of PDGFR-h in HAoSMCs <sup>116, 117</sup>

The proapoptotic activity of sorafenib is significantly enhanced when combined with chemotherapy and signal transduction inhibitors, such as the mTOR inhibitor <sup>117-119</sup>. The full clinical activity of sorafenib may therefore be manifest in combination with chemotherapy and/or signal transduction inhibitors targeting other pathways important in tumor cell growth and survival <sup>120-122</sup>.



**Figure (4):** Sorafenib targeting dysregulated signals in tumor cell, Endothelial (vascular or lymphatic cell) or pericyte<sup>116</sup>

***Approvals and recommended doses*<sup>123,124</sup> (European medical agency "EMA" product information & Drug bank)**

It is approved for for the treatments of hepatocellular carcinoma (HCC), and of advanced renal cell carcinoma in patients who have failed prior interferon-alpha or interleukin-2 based therapy or are considered unsuitable for such therapy.

The recommended dose of sorafenib in adults is 400 mg (two tablets of 200 mg) twice daily (equivalent to a total daily dose of 800 mg).

It is recommended that sorafenib should be administered without food or with a low or moderate fat meal.

Management of suspected adverse drug reactions may require temporary interruption or dose reduction of sorafenib therapy. When dose reduction is necessary, the sorafenib dose should be reduced to two tablets of 200 mg once daily.

***Main toxicities related to sorafenib***

Sorafenib dose received by patients involved in phase I trials (n=197) ranged from 100 bid to 800 bid. The rate of drug-related adverse reactions increased with higher doses of sorafenib. The most common drug-related adverse events representing  $\geq 1/10$  of all experienced AEs are: lymphopenia, lymphophosphatemia, haemorrhage, GIT disorders (nausea, diarrhea), skin and subcutaneous tissue disorder (rash, alopecia, hand foot syndrome) and general fatigue and pain (including mouth, abdominal, bone pain).<sup>123</sup>

***Summary of pharmacokinetic properties (EMA product information & Drug Bank)<sup>123,124</sup>.***

***Absorption, distribution, metabolism and elimination***

After administration of sorafenib tablets the mean relative bioavailability is 38 - 49 % when compared to an oral solution. Following oral administration sorafenib reaches peak plasma concentrations in approximately 3 hours. When given with a high-fat meal sorafenib absorption was reduced by 30 % compared to administration in the fasted state. Steady state plasma sorafenib concentrations are achieved within 7 days, with a peak to trough ratio of mean concentrations of less than 2. The elimination half-life of sorafenib is approximately 25 - 48 hours. Sorafenib is metabolised primarily in the liver and undergoes oxidative metabolism, mediated by CYP 3A4, as well as glucuronidation mediated by UDP-glucuronosyltransferases 1A9 (UGT1A9).

There are no clinically relevant differences in pharmacokinetics between Caucasian and Asian subjects. No relationship was observed between sorafenib exposure and renal function in subjects with normal renal function, mild, moderate or severe renal impairment. No data is available in patients requiring dialysis. The PK of sorafenib in Child-Pugh A and B non-HCC patients were similar to the PK in healthy volunteers. There are no data for patients with Child-Pugh C (severe) hepatic impairment.

## Summary of risks of drug interactions

Caution is recommended when administering sorafenib with compounds that are metabolised/eliminated predominantly by the UDPglucuronosyltransferases 1A1 (UGT1A1) (e.g. irinotecan) or UGT1A9 pathways. Clinical pharmacokinetic interactions of sorafenib with Cytochrome P450 3A4 (CYP3A4) inhibitors are unlikely.

Sorafenib is neither an inhibitor nor an inducer of these cytochrome P450 isoenzymes. Therefore, clinical pharmacokinetic interactions of sorafenib with substrates of these enzymes are unlikely to happen.

### **b- Everolimus**

✦ *The main characteristics of everolimus are mentioned in EMA product information<sup>125</sup>.*

Everolimus (AFINITOR™), an inhibitor of mTOR, is an antineoplastic agent. Everolimus is a selective mTOR inhibitor. As a result, it is a potent inhibitor of the growth and proliferation of tumour cells, endothelial cells, fibroblasts and blood-vessel-associated smooth muscle cells and has been shown to reduce glycolysis in solid tumours *in vitro* and *in vivo*.

✦ *Approvals and recommended doses*

Everolimus is indicated for the treatment of unresectable or metastatic, well- or moderately-differentiated neuroendocrine tumours of pancreatic origin in adults with progressive disease. It is also indicated for the treatment of breast cancer.

Moreover, everolimus is indicated for the treatment of patients with advanced renal cell carcinoma, whose disease has progressed on or after treatment with VEGF-targeted therapy.

The recommended dose is 10 mg everolimus once daily. Treatment should continue as long as clinical benefit is observed or until unacceptable toxicity occurs. If a dose is missed, the patient should not take an additional dose, but take the usual prescribed next dose. Management of severe and/or intolerable suspected adverse reactions may require dose alterations.

Everolimus should be administered orally once daily at the same time every day, consistently either with or without food.

No dose adjustment is required in patients older than 65 years and in patients with renal impairment.

### ***Main toxicities related to everolimus***

The incidence of stomatitis, anemia, asthenia, fatigue, and rash were the most common AEs reported with everolimus therapy.

Other adverse reactions occurring more frequently with everolimus than with placebo, but with an incidence of <5% include:

- Metabolism and nutrition disorders:
  - o Common: dehydration (1.5%), exacerbation of pre-existing diabetes mellitus (1.1%);
  - o Uncommon: new onset of diabetes mellitus
- Psychiatric disorders: Common: insomnia (3.3%)
- Nervous system disorders: Uncommon: ageusia

- Eye disorders: Common: eyelid oedema (3.3%), conjunctivitis (1.5%)
- Cardiac disorders: Uncommon: congestive cardiac failure
- Vascular disorders: Common: hypertension (1.8%); Not known: haemorrhages
- Respiratory, thoracic and mediastinal disorders: Common: haemoptysis (1.1%)
- Gastrointestinal disorders: Common: abdominal pain (3.6%), dysphagia (2.6%), dyspepsia (2.6%)
- Skin and subcutaneous tissue disorders: Common: hand-foot syndrome (4.7%), nail disorder (4.7%), erythema (3.6%), acneiform dermatitis (3.3%), onychoclasia (2.9%), skin exfoliation (1.8%)
- Renal and urinary disorders: Common: increased daytime urination (1.8%)
- General disorders and administration site conditions: ○ Common: chest pain (1.1%); ○ Uncommon: impaired wound healing

Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoan infections, including infections with opportunistic pathogens. Localised and systemic infections, including pneumonia, other bacterial infections, invasive fungal infections such as aspergillosis or candidiasis, and viral infections including reactivation of hepatitis B virus, have been described in patients taking. Some of these infections have been severe (e.g. leading to respiratory or hepatic failure) and occasionally fatal.

- Laboratory findings

Clinical chemistry abnormalities were reported in the majority of patients receiving everolimus therapy, with increases in cholesterol, triglycerides, gamma glutamyltransferase, glucose, creatinine, and alkaline phosphatase, and decreases in phosphate being seen in >30% of patients. The majority of grade 3 abnormalities were increased glucose, increased gamma glutamyltransferase, decreased phosphate, and increased cholesterol. Haematologic abnormalities were common with decreases in red cells, white cells, and platelets being noted in >10% of patients.

### **Summary of pharmacokinetic properties (EMA product information)<sup>125</sup>**

#### *Absorption, distribution, metabolism and elimination*

In patients with advanced solid tumours, peak everolimus concentrations (C<sub>max</sub>) are reached at a median time of 1 hour after daily administration of 5 and 10 mg everolimus under fasting conditions or with a light fat-free snack. Food, however, had no apparent effect on the post absorption phase concentration-time profile. To minimize variability, everolimus should either be consistently taken with food, or consistently taken without it. Everolimus is a substrate of CYP3A4 and Glycoprotein P (PgP). The mean elimination half-life of everolimus is approximately 30 hours. Steady-state was achieved within one to two weeks.

### **Summary of drug interactions with everolimus (EMA product information)<sup>125</sup>**

Everolimus is a substrate of CYP3A4, and also a substrate and moderate inhibitor of PgP. Therefore, absorption and subsequent

elimination of everolimus may be influenced by products that affect CYP3A4 and/or Pgp. *In vitro*, everolimus is a competitive inhibitor of CYP3A4 and a mixed inhibitor of CYP2D6.

#### **IV. Rationale for the combination of everolimus and sorafenib**

*Dual inhibition of RAS-RAF-ERK and PI3K-AKT-mTor signalling pathways in pre-clinical studies*

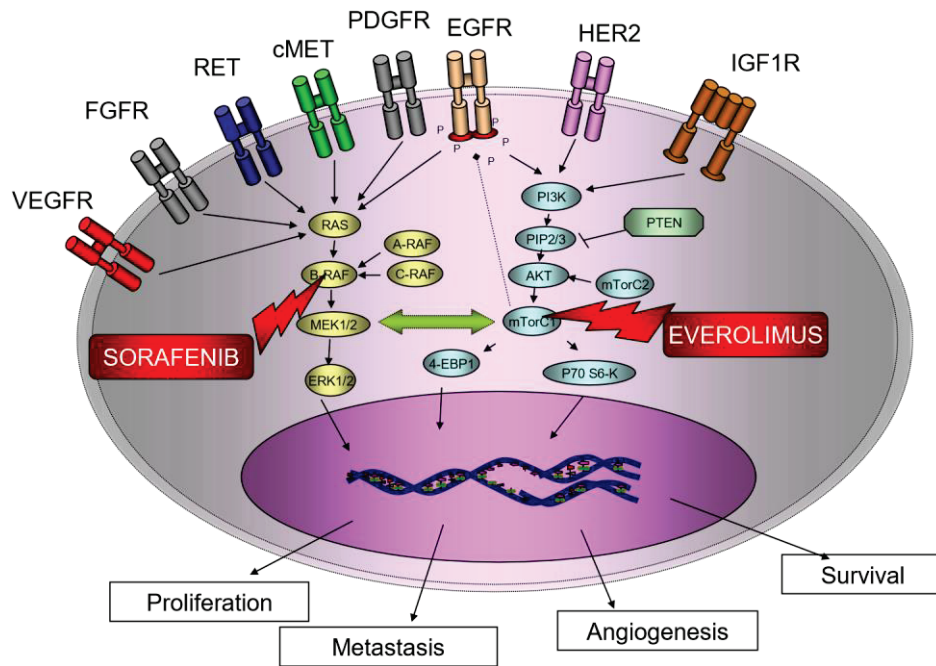
##### *Additive activity of both drugs*

There is a rationale to combine everolimus and sorafenib. Indeed these drugs inhibit 2 important signaling pathways known to interact with each other (**Figure 5**). The cross-talks between RAS-RAF-ERK and PI3K-AKT-mTor were reported so that cancer cells could escape from blockage of a pathway by stimulating the other one. A recent study about gynecological cancers showed that the presence of KRAS mutations was significantly associated with PI3KCA mutations. As a result, the combination has been considered of high interest<sup>126</sup>.

##### *Reversion of resistance to sorafenib*

The mechanisms of resistance to targeted therapies have not been clearly identified. They have been mainly assessed in patients with renal cell carcinoma. AKT pathway activation stimulates VEGF production through hypoxia-inducible factor (HIF)-1 $\alpha$  dependent mechanisms. The ability of mTor inhibitors to downregulate HIF and VEGF makes the combination of these drugs and VEGFR inhibitors interesting for reversing resistance to these treatments. The ability of everolimus and sorafenib combination to reverse resistance to sorafenib is being assessed in an on-going phase 1 trial<sup>127</sup>





**Figure (5):** Rationale for dual inhibition of RAS-RAF-ERK and PI3K/AKT-mTOR signaling pathways using combination of everolimus and sorafenib<sup>128</sup>

*a-Preclinical studies testing the combination of everolimus and sorafenib*

Several in-vitro and in-vivo studies suggested the promising additive anti-proliferative effects of inhibitors of mTOR and RAF signaling pathways as a way to reverse resistance to a single drug<sup>129-131</sup>. A preclinical study involving mice xenografted with osteosarcoma cells treated with everolimus (1 mg/kg/day), sorafenib (5 mg/kg/day) or everolimus + sorafenib confirmed the relevance of this combination<sup>132</sup>. Sorafenib showed a dose-dependent inhibition effect. Everolimus alone was able to affect around 40% of cell proliferation. The combination displayed synergism in the interval of 30-70% of fractions affected (CI<1) and

antagonism at higher doses (CI>1). Sorafenib inhibitory concentration 50 (IC50) was reduced from 2.7 to 1.3 mM in presence of everolimus, with a marked increase in apoptotic cells compared to both single agents. In vivo each treatment strikingly inhibited tumor growth with relative tumor proliferation rate (T/C) values of 0.34 (sorafenib) 0.46 (everolimus) 0.29 (combination). Survival was increased from 12 to 20 days, (p<0.05)<sup>132</sup>.

A second preclinical study confirmed the relevance of this combination. After hepatic implantation of Morris Hepatoma (MH) cells, rats were randomly allocated to everolimus (5 mg/kg, 2×/week), sorafenib (7.5 mg/kg/d), combined everolimus and sorafenib, sequential sorafenib (2 weeks) then everolimus (3 weeks), or control groups. Magnetic resonance imaging quantified tumor volumes. Erk1/2, 4E-BP1, and their phosphorylated forms were quantified by immunoblotting. After 35 days, tumor volumes were reduced by 60%, 85%, and 55%, relative to controls, in everolimus, the combination, and sequential groups, respectively (P < 0.01). Survival was longest in the combination group (P < 0.001). Phosphorylation of 4E-BP1 and Erk1/2 decreased after everolimus and sorafenib, respectively. Angiogenesis decreased after all treatments (P < 0.05). Vessel sprouting was abundant in control tumors, lower after sorafenib, and absent after the combination<sup>130</sup>.

#### *b-Clinical trials of everolimus and sorafenib*

Everolimus + sorafenib regimen has been investigated in at least 14 registered clinical phase 1 or 2 trials including patients with advanced solid cancers or hematological malignancies<sup>133</sup>. In all studies (except one), both

drugs have been assessed on a continuous basis considering addition of each monotherapy regimen would be optimal: once a day for everolimus and twice a day for sorafenib.

In addition to the below listed clinical trials, there are ongoing phase 1 and 2 trials studying everolimus and sorafenib combination in recurrent high-grade Gliomas (still recruiting). Also, the combination was tested on acinar Cell adenocarcinoma of the pancreas; duct cell adenocarcinoma of the pancreas; recurrent pancreatic cancer; stage IV pancreatic cancer in a phase 1 study and a global study to treat patients with advanced hepatocellular carcinoma (completed but no results are published till present)<sup>133</sup>.

In the large majority of previous trials testing the combination, both drugs were given continuously without any real assessment of PK and PD interactions. In a small study involving 13 patients with solid tumors, recommended doses were daily 2.5 mg everolimus and 600 mg sorafenib. Neither grade 4 nor PK interactions were identified<sup>134</sup>. In a Phase II trial of daily 5-mg everolimus and 400-mg sorafenib twice daily in 38 patients with metastatic osteosarcoma, the disease control rate was 63%, but toxicity led to dose reductions or interruptions in 66% patients<sup>135</sup>. Indeed, despite promising signs of efficacy, significant metabolically and clinical toxicities have led some drug industries to abandon this association.

We assume it is possible to identify the optimal doses and dosing schedules of everolimus and sorafenib combinations offering the maximization of benefit/toxicity ratio using modeling of data from an adequately designed trial.

Mathematical models able to describe biological phenomena at different complexity scales by using equations and to simulate the effects induced by changes in experimental conditions, may provide some solutions to this issue <sup>136-140</sup>. Indeed, based on the data of adequately designed clinical trials, mathematical modeling can define the best dose and dosing schedule of drugs using simulations, as demonstrated during the development of everolimus <sup>141</sup>. We assume this tool may be used to identify the optimal doses and dosing schedules of combined study agents based on maximization of the simulated expected benefit/toxicity ratio. However, such a strategy requires the data from adequately designed early-phase trials, called multiparameter phase I trials, where different doses and dosing schedules are investigated together with multiple biological, radiological and clinical parameters. These data are required so the links between doses, dosing schedules, PK, PD, pharmacogenetics, radiological and clinical effects can be quantified by the models.

**Table (1): Previous early phases clinical trials of everolimus and sorafenib**

Studies	Drugs dose ranges (mg/m <sup>2</sup> )	No of pts	Disease site	MTD	Main grade 3-4 adverse events (%)	DLTs	Efficacy ORR (%) PR (%) SD (%) PFS/TTP (months)
Harzstark et al.,2011 <sup>142</sup> .	EVE: 2.5 – 10 mg qd SOR: 200-400 mg bid	20	RCC	EVE: 5 mg qd SOR: 400 mg bid	Hypophosphatemia: 45% Fatigue: 10% Diarrhea: 10% Rash: 10% Lymphoneutropenia: 10% Mucositis: 5% Oedema: 5% Anorexia: 5% Hypokaliemia: 5% Lipase increase: 5%	Grade 4 hyperuricemia, associated with grade 2 gout (n=1),  Grade 3 lipase, associated with grade 2 pancreatitis (n=1)  Grade 3 rash (n=2)	PR: 25%

					Photosensitivity: 5% Pneumonitis: 5%		
Giessinger et al., 2008 <sup>143</sup>	EVE: 2.5-10 mg qd SOR: 400 mg bid	13	RCC	ND	HFS: 17% Pneumonitis: 8% Pleural effusion: 8% Thrombocytopenia: 8%	ND	PR: 58% SD: 30% PD: 8%
Cen et al., 2009 <sup>144</sup>	EVE: 2.5 – 10 mg qd SOR: 400 mg bid	18	RCC	EVE: 10 mg qd SOR: 400 mg bid	ND	Thrombocytopenia (n=1) Pneumonitis (n=1) Pulmonary embolism (n=1)	CR: 6% SD: 47%
Chan et al., 2010 <sup>145</sup>	EVE: 10 mg qd SOR: 200-400 mg bid	9	NET	EVE: 10 mg qd SOR: 200 mg bid	Grade 3 thrombocytopenia Grade 3 hypophosphatemia Grade 3 hypokaliemia Grade 4 hypocalcemia	Grade 3 skin rash (n=2) Grade 3 HFS (n=1) Grade 3 thrombocytopenia (n=1) Grade 3 hypophosphatemia (n=1)	ORR: 100% (5/5)
Finn et al., 2012 <sup>146</sup>	EVE: 2.5-5 mg qd SOR: 400 mg bid	30	HCC	EVE: 2.5 mg qd SOR: 400 mg bid	Thrombocytopenia: 42% Neutropenia: 21%	Grade 3 AST elevation (n=1) Grade 3-4 thrombocytopenia (n=5) Grade 3 hyperbilirubinemia (n=1)	SD: 62.5% (2.5 mg) ORR: 0% SD: 35.7% (5mg)
Nogova et al., 2011 <sup>147</sup>	EVE: 2.5-10 mg qd SOR: 400 mg bid	19	Solid tumors	EVE: 7.5 mg qd SOR: 400 mg bid	Grade 3 upper respiratory tract infection: n=1 Grade 3 leukopenia: n=1 Grade 3 thrombopenia: n=1 Sudden cardiac death probably due to arrhythmia: n=1	None	ND
Waterhouse et al., 2011 <sup>148</sup>	EVE: 35 mg q1w SOR: 400 mg qd – bid	60	RCC	EVE: 35 mg q1w SOR: 400 mg qd	Anemia: 11% Thrombocytopenia: 4% Fatigue: 17% Proteinuria: 9% Diarrhea: 4% High blood pressure: 4% HFS: 3% Atrial fibrillation: 1% Nausea: 1% Rash: 1%	Grade 2 HFS (n=4) Grade 3 proteinuria (n=1)	ORR = 5-8% SD: 61-76%

## V. Analysis of the literature data

### Phase I trial design for solid tumors studies:

Progresses in molecular biology and genetics offer large perspectives in the understanding of tumorigenesis processes and drug development, they also bring new biological and pharmacological issues that should be addressed, so they can be translated into real benefit for treatments of cancer patients. Problems recently raised in the development of novel targeted agents highlight the need for novel strategies. Unlike conventional chemotherapy, no dose-toxicity or dose-efficacy relationships have been identified for most of these novel compound. Despite that, most of the phase 1 trials, which are designed to identify the recommended dose for phase 2 trials (RP2D), rely on dose escalation and toxicity-based traditional endpoints. This strategy is particularly inappropriate for compounds able to reach maximal target inhibition at low non-toxic doses.

As a consequence, new drug development strategies meant to identify the best doses and dosing schedules of novel targeted agents have been proposed, among them, determination of the OBD appears promising. Indeed, identification of the minimal dose associated with optimal biological effect through measurement of target inhibition and pharmacokinetics analysis might be a good alternative for defining the recommended phase II trial dose. However, although OBD is an attractive endpoint for defining the RP2D of novel targeted agents, there is no data about the actual relevance in terms of clinical efficacy of this endpoint.

## VI. Thesis objectives

The work of the present thesis is divided in two major parts aiming at studying the optimisation of early phase clinical trials development in oncology:

*The first part is the analysis of the preliminary results of EVESOR study.* EVESOR may be a ‘proof of concept’ study of a new type of ‘multiparameter trial’ meant to optimize the information provided by early-phase trials. First, data about the safety of different dosing schedules and doses of both combined drugs was extracted from this trial. Noteworthy, several recommended doses for Phase II trials may be identified on the different dosing schedules. Moreover, multiples PK, PD, and radiological and clinical data were collected, so the relationships between these parameters and doses/dosing schedules might be understood and quantified using mathematical modeling.

*The second part is an extensive* research analysis of the literature review to examine if the optimal biological doses of molecular targeted therapies defined during early phase trials were useful during subsequent drug development. In another word, the litterature review addressed the question of wether OBDs defined during early phase trials were found to be clinically effective in subsequent phase II and III trials, and useful for eventual further approval. We assessed differences between the OBD defined in early phase trials, and the eventual clinical effective doses, defined as the doses approved by FDA, if any; or the doses associated with positive outcomes in randomized phase III trials based on the primary endpoint.

## Aim of the Work

The specific aim of the current thesis is about the optimisation of early phase clinical trials development in oncology. This was based on the analysis of the preliminary data of EVESOR study in which the principal objective was to determine the effect of the different dose and treatment schedule of administration on PK and PD of sorafenib and everolimus combination. In parallel to EVESOR preliminary analysis, an extensive research analysis of the literature review was conducted to investigate the utility of the optimal biological dose defined in early phases and its impact during subsequent drug development.

In general, everolimus-sorafenib combination may be very effective as suggested by preliminary outcomes of on-going trials, but the significant toxicity related to daily administration of both drugs may compromise further development. The drug dose-intensity in EVESOR trial, should be associated with lower risk of adverse events. We believe determination of the best administration sequences; dosing schedules (intermittent dosings) and doses of both drugs, based on this trial and modeling/simulations, may enable maximization of benefit/toxicity ratio. However, we are presenting the preliminary analysis based on which a PK-PD model will be constructed on a later phase in order to determine the recommended phase 2 dose. We are also based on correlation studies to explain the relationship between drug exposure, toxicity and tumor biomarkers to define the best treatment schedule to be given to the patients.



On the other hand, the concept of optimising drug development of early phase oncology trials was further supported by research analysis of the literature review to examine the clinical relevance of optimal biological dose that appears to be a promising endpoint for defining the RP2D of novel molecular targeted therapies in early-phase clinical trials.

## Patients and Methods

### *EVESOR Study*

#### **I. Study Design and Treatment Regimens**

This phase I, academic non-randomized study was conducted at the Centre Hospitalier Lyon-Sud (Lyon, France) in May, 2013 and at the Centre Léon Bérard (Lyon, France) in February, 2014. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization, Harmonized Tripartite Guidelines for Good Clinical Practice. It was approved by an independent ethics committee and by the French authority for clinical trials, Agence National de la Sécurité des Médicaments.

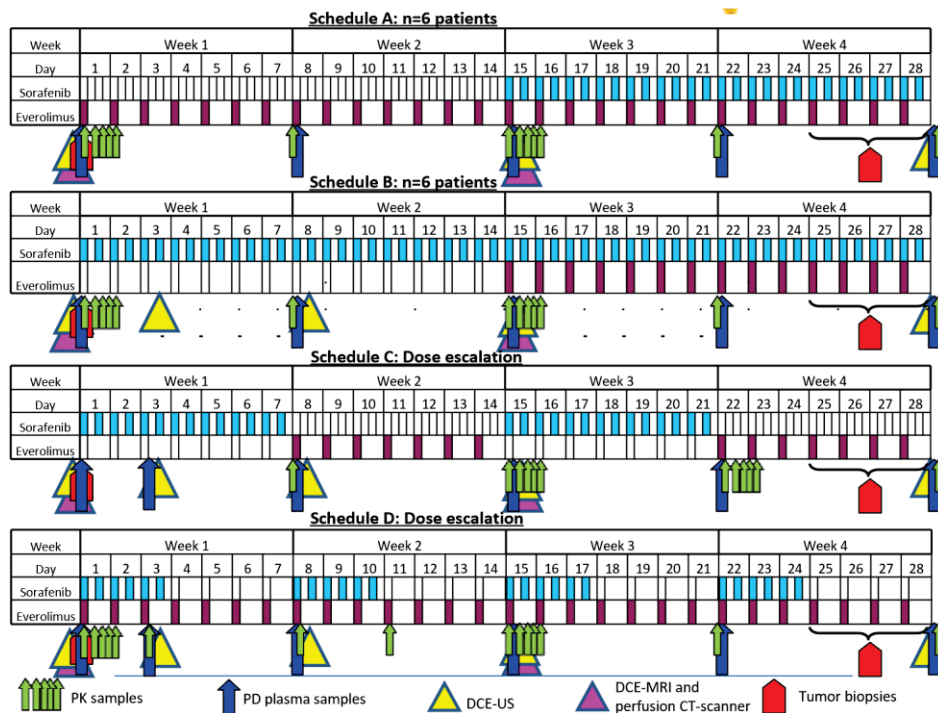
Everolimus (AFINITOR™) and sorafenib (NEXAVAR™) combination was given according to four different administration schedules.

- Schedule A and B (everolimus 5 mg qd and sorafenib 200 mg bid after 2 week run-in periods of either drug), currently investigated in ongoing phase I trials, will be repeated with 6 patients enrolled in both arms. This repetition is intentional in order to correlate our PK results with those to be reported by other authors, to ensure validity of our PK and PD assays. In addition, because the combination will be started after 2 week run-in periods of everolimus alone (schedule A) or sorafenib alone (schedule B) respectively, these arms will enable assessment of PK and PD interactions between the two drugs.

- Schedule C (alternating everolimus qd and sorafenib bid every other week with dose-escalation) was selected because sorafenib administration every other week was shown to be safe in a phase 1

trial <sup>149</sup> and because one week was the time required to observe steady state concentrations of both drugs <sup>150,151</sup>.

- Schedule D (sorafenib bid 3 day-on, 4 day-off; everolimus qd; doseescalation) was selected because 3 consecutive day treatment has been recognized as the time required for tumor vessel normalization with most of anti-angiogenic drugs. As a result, we assume tumor exposure to everolimus will be improved during this normalization window. In all arms, a cycle lasted for 28 days <sup>152</sup>.



**Figure (6): Design of EVESOR trial during cycle 1.** PK: Pharmacokinetic samples; PD: Pharmacodynamic samples, including peripheral blood mononuclear cells (PBMCs), soluble markers of angiogenesis (VEGF, VEGFR1, VEGFR2) and circulating tumor nucleic acids; DCE-US: dynamic contrast enhanced ultrasound; DCE-MRI: dynamic contrast enhanced magnetic resonance imaging.

## **II. Treatment Plan**

### ***1. Allocation to Treatment Schedules***

Patient assignment proceeded as follows:

All schedules began at dose level 1. (everolimus 5 mg qd and sorafenib 200 mg bid). Drug dosing levels are described in Table 2 below. Schedules A, B, C and D were open concurrently. Patient allocation to one of these four administration schedules were determined according to availability of open slots within each schedule. Patients were assigned consecutively to fill each schedule (from A to D) with available slots. Patients will not be selected to any specific schedule based on their characteristics, their preference or physician choice. Dose level 1 of schedule A will be filled first, followed by dose level 1 of schedule B, then followed by dose level 1 of schedule C and schedule D.

**Table (2): Drug dosing and drug dosing levels in the EVESOR trial.**

Schedule	Drug name	Schedule of administration	Dose levels (mg)						No. of pts per schedule	Cycle length
			-2	-1	1	2	3	4		
A	Everolimus	Once a day starting day 1; Continuously	2.5	2.5	5				6	4 weeks (28 days)
	sorafenib	Twice a day starting day 15; Continuously	200 once a day	200	200					
B	Everolimus	Once a day starting day 15; Continuously	2.5	2.5	5				6	
	sorafenib	Twice a day starting day 1; Continuously	200 once a day	200	200					
C	Everolimus	Once a day starting day 8; every other week	2.5	2.5	5	5	7.5	10	Dose escalation	
	sorafenib	Twice a day starting day 1; every other week	200 once a day	200	200	400	400	400		
D	Everolimus	Once a day starting day 1; Continuously	2.5	2.5	5	5	7.5	10	Dose escalation	
	sorafenib	Twice a day starting day 1; 3 days-on; 4 days-off	200 once a day	200	200	400	400	400		

## 2. Dose Limiting Toxicity (DLT)

### - Definition of Dose-Limiting Toxicity

Patients were evaluated for Dose-limiting toxicity (DLT) during the first 28-day cycle. DLT is defined based on adverse events observed in cycle 1 that are possibly, probably or definitively related to study drugs.

DLT is defined as:

### - Hematologic DLTs

- Absolute neutrophil count (ANC) < 0.5 x 10<sup>9</sup>/L for 7 or more consecutive days.

- Febrile neutropenia ( $ANC < 1.0 \times 10^9/L$  and fever  $\geq 38.5^\circ C$ ).
- Platelets  $< 25 \times 10^9/L$  or thrombocytopenic bleeding (i.e. platelets  $< 50 \times 10^9/L$  and associated with clinically significant bleeding).
- Non-hematologic DLTs
  - Hand-foot syndrome (HFS)  $\geq$  Grade 3 despite management (defined in appendix)
  - Other Common terminology criteria of adverse events (CTCAE)  $\geq$  Grade 3 toxicity thought to be treatment related, despite adequate medical intervention as judged by the investigator, excluding toxicities that do not pose a safety risk (e.g., alopecia, asymptomatic hypophosphatemia, hypocalcemia or hypomagnesemia)
  - Treatment-related toxicities that result in failure to receive at least 80% of the planned doses of either drug in cycle 1 (i.e. at least 22 of 28 doses of either drug on schedule A or B or D; at least 44 of 56 sorafenib doses on schedule A or B, at least 11 of 14 doses of either drug on schedule C, at least 9 of 12 sorafenib doses on schedule D) despite maximal (as judged by the investigator) supportive care measures
  - Inability to resume dosing for cycle 2 at the current dose level within 14 days (i.e. by cycle 1 day 43) due to treatment-related toxicity

### **3. Dose escalation rules**

In *schedules C and D*, 3+3 dose-escalations were used to determine the MTDs, defined as the highest doses (which may be different in each arm) at which 2 patients out of 6 experienced dose-limiting toxicities. Treatments continued in all patients until disease progression (determined

with the Response Evaluation Criteria in Solid Tumors [RECIST], version 1.1 or clinically), withdrawal of consent, or unacceptable toxicity as explained in Table 3<sup>153</sup>. The selection of dose levels was defined by consensus between investigators, data safety and monitoring board, and Novartis (France), with safety purposes based on the literature data.

Patients were evaluated for DLT during the first 28-day cycle. All three patients treated on a dose level (at any given schedule) were observed for at least 28 days (one cycle) for any toxicity, and assessed for any DLT, before 3 other patients can be entered on the same dose level or on next dose level.

For each schedule, RP2D is the dose at which  $\leq 1/6$  encountered DLT.

**Table (3):** Conventional 3+3 dose escalation rule for schedules C and D

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level
$\geq 2$	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered) <b>for that schedule</b> . Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. <ul style="list-style-type: none"> <li>• If 0 of these 3 patients experience DLT, proceed to the next dose level.</li> <li>• If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose <b>for that schedule</b>. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.</li> </ul>
$\leq 1$ out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose <b>for that schedule</b> . At least 6 patients must be entered at the recommended phase 2 dose.



### **III. Patient selection**

The selection of patients was carefully done by the principal investigator according to inclusion and exclusion criteria following study protocol. The selection took place at the Centre Hospitalier Lyon-Sud (Lyon, France) in May 2013 and at the Centre Léon Bérard (Lyon, France) in February 2014.

### **IV. Safety parameters**

Descriptive statistical analysis of all adverse events and serious adverse events was performed based on data collected and recorded in case report form (CRF). Treatment related adverse events for continuous schedules (Arm A and Arm B) and intermittent schedules (C and D) were calculated as well as frequency of adverse events in different treatment. Analysis of serious adverse events (SAEs) by system organ class (SOC) was also performed. Safety evaluations were conducted by the principal investigator at baseline and on weeks 1, 2, 3, and 4 of cycle 1; then, every other week on subsequent cycles. Safety evaluation is explained in appendix.

#### **Pharmacokinetic sampling analysis**

Sampling times are presented in Table 4. Concentrations of everolimus and sorafenib were quantified in serum and plasma samples, respectively. Samples were stored at  $-80^{\circ}\text{C}$  until analysis. For both drugs, fully validated liquid chromatography MS/MS (LC-MS/MS) assays were used for drug determinations as described by Moes et al., 2012<sup>154</sup>. The concentrations ranged from 2.2 to 43.7  $\mu\text{g/L}$  for everolimus and from 5 to 7260  $\mu\text{g/L}$  for sorafenib. five mL of venous blood were collected by

venipuncture, through a heparin lock or through a central line into a EDTA vacutainer at the time points specified below in Table 6. Samples must be kept in ice bath immediately after collection. Within 30 minutes of collection, each blood sample should be divided in two parts. For the quantification of sorafenib, 4 mL should be centrifuged at 4°C and 3000 rpm for 10 minutes. One mL of whole blood will be used for the quantification of everolimus. In both cases, the plasma portion and the whole blood portion was transferred in equal portions to duplicate properly labeled polypropylene tubes and frozen at -80°C in an upright position within 1 hour of collection. Each tube will be labeled with study number, patient initials, patient’s study number, date and time of drug administration, and date and time of sample collection. All labels will be affixed to the test tubes properly and prior to freezing. A PK sample log will be completed at the site.

**Table (4): Pharmacokinetic sampling schedule.**

Schedule	Study drug	Cycle	PK Sampling Time-Points		No. of PK samples
			Cycle Day	Time	
Schedule A	Everolimus	1	Day 1 + 2	Before drug dosing	8
				0.5, 1, 2, 4, 6, 8 and 24 h after dosing	
			Day 8	Before drug dosing	1
			Day 15 + 16	Before drug dosing	8
				0.5, 1, 2, 4, 6, 8 and 24 h after dosing	
			Day 22	Before drug dosing	1

		2+	Day 1 <sup>^</sup>	Before drug dosing	1+	
	sorafenib	1	Day 15 + 16	Before drug dosing	8	
				0.5, 1, 2, 4, 6, 8 and 24 h after dosing		
			Day 22	Before drug dosing	1	
		2+	Day 1 <sup>^</sup>	Before drug dosing	1+	
Schedule B	Everolimus	1	Day 15 + 16	Before drug dosing	8	
						0.5, 1, 2, 4, 6, 8 and 24 h after dosing
				Day 22	Before drug dosing	1
			2+	Day 1 <sup>^</sup>	Before drug dosing	1+
	sorafenib	1	Day 1 + 2	Before drug dosing	8	
						0.5, 1, 2, 4, 6, 8 and 24 h after dosing
				Day 8		Before drug dosing
			Day 15 + 16	Before drug dosing	8	
						0.5, 1, 2, 4, 6, 8 and 24 h after dosing
			Day 22	Before drug dosing	1	
		2+	Day 1 <sup>^</sup>	Before drug dosing	1+	
Schedule C	Everolimus	1	Day 8	Before drug dosing	1	
			Day 22 + 23	Before drug dosing	8	
				0.5, 1, 2, 4, 6, 8 and 24 h after dosing		
		2+	Day 1 <sup>^</sup>	Before drug dosing	1+	
Schedule	Study drug	Cycle	PK Sampling Time-Points		No. of PK samples	
			Cycle Day	Time		
	sorafenib	1	Day 8	Before drug dosing	1	

			Day 15 + 16	Before drug dosing	8	
				0.5, 1, 2, 4, 6, 8 and 24 h after dosing		
			Day 22	Before drug dosing	1	
		2+	Day 1^	Before drug dosing	1+	
Schedule D	Everolimus	1	Day 1 + 2	Before drug dosing	8	
				0.5, 1, 2, 4, 6, 8 and 24 h after dosing		
			Day 3	Before drug dosing	1	
			Day 8	Before drug dosing	1	
			Day 11	Before drug dosing	1	
			Day 15 + 16	Before drug dosing	8	
				0.5, 1, 2, 4, 6, 8 and 24 h after dosing		
			Day 22	Before drug dosing	1	
			2+	Day 1^	Before drug dosing	1+
		sorafenib	1	Day 1 + 2	Before drug dosing	8
					0.5, 1, 2, 4, 6, 8 and 24 h after dosing	
				Day 3	Before drug dosing	1
				Day 8	Before drug dosing	1
				Day 11	Before drug dosing	1
	Day 15 + 16			Before drug dosing	8	
				0.5, 1, 2, 4, 6, 8 and 24 h after dosing		
	Day 22			Before drug dosing	1	
		2+	Day 1^	Before drug dosing	1	

## **V. Pharmacokinetic data analyses**

### ***1. Selection of structural models***

Data were analyzed with a population approach (based on nonlinear mixed effect model methodology). Compartmental PK models for everolimus and sorafenib were fit to the drug concentration versus time data from groups with the same dosing history (amounts and schedules for both drugs). Non-linear mixed effect modeling (NONMEM 7, ICON Development Solutions, Ellicott City, MD) was performed to estimate population pharmacokinetic parameters of sorafenib and everolimus (fixed/typical values and random/inter-individual variability) and to identify potential covariates that may explain inter-individual variability in the parameters. Structural models of both drugs were selected with the first order conditional estimation method, with an interaction option (FOCEI). Covariate screening was carried out to identify potential significant covariates of the models, based on stepwise addition of each covariate to the structural model. Two structural compartmental PK models were selected that best fit the data: a 1 compartment model for sorafenib, and a 2-compartment model for everolimus. The models were evaluated during model building, based on a successful termination of the run, goodness of fit plots, and the precision of the estimated parameters. Graphical diagnostics were assessed with Xpose version 4 (Uppsala University, Sweden). Exponential error models were used in both PK models to explain inter-individual variabilities of the main pharmacokinetic parameters, which included the apparent oral clearance (CL/F), the central volume of distribution ( $V_{d_{\text{central}}}$ ), and the absorption rate constant (Ka). Residual error (the difference between predicted and observed concentrations) variability

and inter-occasion variabilities (IOV), due to different administration schedules) were also evaluated in the two structural models.

Based on empirical Bayesian estimates of the individual parameters, the following secondary pharmacokinetic parameters were **assessed** for the two drugs: areas under the concentration versus time curves within the dosing interval ( $AUC_T$ ), peak concentrations ( $C_{max}$ ), and the time at which  $C_{max}$  occurred ( $T_{max}$ ).

## ***2. Qualification of the models with a Visual Predictive Check (VPC)***

The final structural models of sorafenib and everolimus were qualified with **goodness-of-fit plots and** visual predictive checks (VPC) of simulations of 500 samples from virtual patients. The distributions of the simulated concentration-time courses were compared with the distributions of the observed values from the original datasets. Differences and overlaps between the simulated and original distributions provided information on the accuracy of the identified models.

## ***3. Analysis of PK interactions***

To identify significant differences ( $p < 0.05$ ) between PK parameters of the two drugs in the different administration schedules, the median values of the estimated **post-hoc** pharmacokinetics parameters were compared with **non-parametric Wilcoxon tests for unpaired ttests**, provided in R software (version 3.1.3).

## **VI. Pharmacodynamics (PD) analyses**

The EVESOR trial included assessments of multiple parameters to enrich the final model, which was built to define the best doses and dosing

schedules for the two drugs. Inhibitions of PI3K–AKT–mTor and RAS–RAF–ERK signaling pathways were serially assayed in PBMCs by quantitative assessment of the expression levels of Total **Protein kinase B** (AKT Total), Phosphorylated **Protein kinase B** (pAKT), phosphorylated p70 Ribosomal Protein S6 Kinase (p70-S6K) pS6K, Total Extracellular Signal Regulated Kinase ERK1/2 Proteins (ERK Total), and Phosphorylated Extracellular Signal Regulated Kinase ERK1/2 Proteins (pERK) with ELISA kits from Invitrogen (Carlsbad, CA) (**Table 5**). vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor 1 (VEGFR1) and vascular endothelial growth factor receptor 2 (VEGFR2) were measured serially in the serum with human ELISA kits from Abcam (Cambridge, UK)..

**Table (5): Sampling strategies of peripheral blood mononuclear cells (PBMCs) and soluble markers of angiogenesis for each dosing schedule**

Schedule	Cycle	Sampling Time-Points: Cycle Day (samples were acquired before the study drug dose was administered)	PBMCs	Soluble markers of angiogenesis
Schedule A and B	1	Day 1	X	X
		Day 8	X	X
		Day 15	X	
		Day 22	X	
	2	Day 1	X	X
Schedule C and D	1	Day 1	X	X
		Day 3	X	X
		Day 8	X	X
		Day 15	X	
		Day 22	X	
	2	Day 1	X	X

Fourteen (14) mL of venous blood was collected on 2 EDTA tubes (purple top) before rapid transportation to the laboratory for PBMC isolation. PBMCs was isolated from total blood by FICOLL density gradient centrifugation. PBMCs will be stored at -80°C before experiments.

Quantitative Estimation of Treatment-Mediated Changes in the Phosphorylation of Extracellular Signal Regulated Kinase ERK1/2 Proteins (ERK total) and (ERK phosphorylated) in PBMC using ELISA assays



ERK1/2 Proteins (total) and (phosphorylated) was determined in PBMC according to the method described by Manuel Hidalgo et al., 2011

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### ***Principle***

The Invitrogen ERK1/2 (Total) or (pTpY185/187) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for ERK1/2 (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. During the first incubation, the ERK1/2 antigens bind to the immobilized (capture) antibody. After washing, an antibody specific for both ERK1 and ERK2 is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized ERK1/2 proteins captured during the first incubation. After removal of excess detection antibody, a horseradish peroxidase labeled Anti-Rabbit IgG (Anti-Rabbit IgG HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove all the excess Anti-Rabbit IgG HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of ERK1/2 present in the original specimen.

### ***Protocol of cell extraction:***

1. Cells were collected in Phosphate Buffered Saline (PBS) by centrifugation
2. Cells were washed twice with cold PBS.

3. The supernatant was removed and discarded and the cell pellet was collected (At this point the cell pellet can be frozen at  $80^{\circ}\text{C}$  and lysed at a later date).
4. The cell pellet was lysed in the appropriate extraction buffer for 30 minutes on ice with vortexing at 10 minute intervals.
5. Extracts were transferred to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at  $4^{\circ}\text{C}$ .
6. The clear lysate was aliquoted to clean microfuge tubes of 2 ml and realize a second tube of 20  $\mu\text{L}$ . These samples are ready for assay. Lysates can be stored at  $-80^{\circ}\text{C}$ .

***Sample pre-treatment:***

Serum was removed from the clot or cells as soon as possible after clotting and separation. Samples were aliquoted and stored frozen at  $20^{\circ}\text{C}$  to avoid loss of bioactive sample Treatment Buffer: When cells are lysed with Cell Extraction Buffer, incubate each sample and control with an equal volume of Sample Treatment Buffer on ice for 20 minutes.

***Standard preparation***

- 1- Reconstitute ERK1/2 Standard with Standard Diluent Buffer.
- 2- Mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 2000 pg/mL ERK1/2.
- 3- Use standard within 1 hour of reconstitution.
- 4- Add 0.25 mL of Standard Diluent Buffer to each of 6 tubes labeled 1000, 500, 250, 125, 62.5, and 31.2 pg/mL ERK1/2. 3.

*Assay Method*

1. Add 100  $\mu$ L of the Standard Diluent Buffer to zero well.
2. Add 100  $\mu$ L of standards, samples or controls to the appropriate microtiter wells.
3. Cover plate with plate cover and incubate for 2 hours at room temperature. Alternatively, the plate may be incubated overnight at 4°C
4. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times
5. Add 100  $\mu$ L Anti-Rabbit IgG HRP Working Solution to each well except the chromogen blank(s)
6. Add 100  $\mu$ L of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue. Incubate for 30 minutes at room temperature and in the dark.
7. Add 100  $\mu$ L of Stop Solution to each well. Tap side of plate gently to mix.
8. Read the absorbance of each well at 450 nm.

Quantitative Estimation of Treatment-Mediated Changes in the Phosphorylation of AKT Proteins (AKT total) and (AKT phosphorylated) in PBMC using ELISA assays

AKT Proteins (total) and (phosphorylated) was determined in PBMC according to the method described by Cataldo Bianco et al.,2006

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### *Principle*

The Invitrogen AKT (Total) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for AKT (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. During the first incubation, the AKT antigen binds to the immobilized (capture) antibody. After washing, a biotin-conjugated monoclonal antibody, specific for AKT Total, is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized AKT protein captured during the first incubation. After removal of excess detection antibody, horseradish peroxidase-labeled streptavidin (SAV-HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove all the excess SAV-HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of AKT present in the original specimen.

### *Protocol of cell extraction*

1. Cells were collected in Phosphate Buffered Saline (PBS) by centrifugation
2. Cells were washed twice with cold PBS.
3. The supernatant was removed and discarded and the cell pellet was collected (At this point the cell pellet can be frozen at -80°C and lysed at a later date).
4. The cell pellet was lysed in the appropriate extraction buffer for 30 minutes on ice with vortexing at 10 minute intervals.

5. Extracts were transferred to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
6. The clear lysate was aliquoted to clean microfuge tubes of 2 ml and realize a second tube of 20  $\mu$ L. These samples are ready for assay. Lysates can be stored at -80°C.

***Sample pre-treatment:***

Serum was removed from the clot or cells as soon as possible after clotting and separation. Samples were aliquoted and stored frozen at 20°C to avoid loss of bioactive

Sample Treatment Buffer: When cells are lysed with Cell Extraction Buffer, incubate each sample and control with an equal volume of Sample Treatment Buffer on ice for 20 minutes.

***Standard preparation***

1. Reconstitute AKT (total) or (phosphorylated) Standard with Standard Diluent Buffer.
2. Mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 20 ng/mL AKT. Use standard within 1 hour of reconstitution.
3. Add 0.25 mL of Standard Diluent Buffer to each of 6 tubes labeled 10, 5, 2.5, 1.25, 0.6, and 0.3 ng/mL AKT.

***Assay Method***

1. Add 100  $\mu$ L of the Standard Diluent Buffer to zero well.
2. Add 100  $\mu$ L of standards, samples or controls to the appropriate microtiter wells.

3. Cover plate with plate cover and incubate for 2 hours at room temperature. Alternatively, the plate may be incubated overnight at 4°C
4. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times
5. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s).
6. Add 100 µL of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue. Incubate for 30 minutes at room temperature and in the dark.
7. Add 100 µL of Stop Solution to each well. Tap side of plate gently to mix.
8. Read the absorbance of each well at 450 nm

Quantitative Estimation of Treatment-Mediated Changes in the phosphorylated p70 Ribosomal Protein S6 Kinase (p70-S6K) in PBMC using ELISA assays p70-S6K was determined in PBMC according to the method

described by Hartmann et al.,2013<sup>157</sup>

### ***Principle***

The Invitrogen kit Protein S6 Kinase (p70-S6K) is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for p70-S6K (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. During the first incubation, the p70-S6K antigens bind to the immobilized (capture)

antibody. After washing, an antibody specific for p70-S6K is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized p70-S6K proteins captured during the first incubation. After removal of excess detection antibody, a horseradish peroxidase labeled Anti-Rabbit IgG (Anti-Rabbit IgG HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove all the excess Anti-Rabbit IgG HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of p70-S6K present in the original specimen.

***Protocol of cell extraction:***

1. Cells were collected in Phosphate Buffered Saline (PBS) by centrifugation
2. Cells were washed twice with cold PBS.
3. The supernatant was removed and discarded and the cell pellet was collected (At this point the cell pellet can be frozen at  $-80^{\circ}\text{C}$  and lysed at a later date).
4. The cell pellet was lysed in the appropriate extraction buffer for 30 minutes on ice with vortexing at 10 minute intervals.
5. Extracts were transferred to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at  $4^{\circ}\text{C}$ .
6. The clear lysate was aliquoted to clean microfuge tubes of 2 ml and realize a second tube of 20  $\mu\text{L}$ . These samples are ready for assay. Lysates can be stored at  $-80^{\circ}\text{C}$ .

***Sample pre-treatment:***

Serum was removed from the clot or cells as soon as possible after clotting and separation. Samples were aliquoted and stored frozen at 20°C to avoid loss of bioactive sample Treatment Buffer: When cells are lysed with Cell Extraction Buffer, incubate each sample and control with an equal volume of Sample Treatment Buffer on ice for 20 minutes.

***Standard preparation***

- 1- Reconstitute p70-S6K Standard with Standard Diluent Buffer.
- 2- Mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 2000 pg/mL p70-S6K
- 3- Use standard within 1 hour of reconstitution.
- 4- Add 0.25 mL of Standard Diluent Buffer to each of 6 tubes labeled 1000, 500, 250, 125, 62.5, and 31.2 pg/mL p70-S6K.

***Assay Method***

1. Add 100 µL of the Standard Diluent Buffer to zero well.
2. Add 100 µL of standards, samples or controls to the appropriate microtiter wells.
3. Cover plate with plate cover and incubate for 2 hours at room temperature. Alternatively, the plate may be incubated overnight at 4°C
4. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times
5. Add 100 µL Anti-Rabbit IgG HRP Working Solution to each well except the chromogen blank(s)



6. Add 100  $\mu$ L of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue. Incubate for 30 minutes at room temperature and in the dark.
7. Add 100  $\mu$ L of Stop Solution to each well. Tap side of plate gently to mix.
8. Read the absorbance of each well at 450 nm.

Quantitative Estimation of Treatment-Mediated Changes in the vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor 1 (VEGFR1) and vascular endothelial growth factor receptor 2 (VEGFR2) in PBMC using ELISA assays

VEGF, VEGFR1 and VEGFR2 were determined in PBMC according to the method described by Chen et al., 2014 <sup>158</sup> & Titin andri Wihastuti et al., 2014 <sup>159</sup>

***Principle***

VEGFA, VEGFR1, VEGFR2 specific antibodies have been precoated onto 96-well plates. Standards and test samples are added to the wells and then incubated at room temperature. After washing, a Biotinconjugated anti-Human VEGFA VEGFR1, VEGFR2 detection antibody is added then incubated at room temperature. Following washing Streptavidin-HRP conjugate is added to each well, incubated at room temperature then again washed. TMB substrate is added and then catalyzed by HRP to produce a blue color product that changes into yellow after addition of an acidic stop solution. The density of yellow coloration is directly proportional to the amount of VEGFA captured on the plate.

***Protocol of cell extraction***

1. Cells were collected in Phosphate Buffered Saline (PBS) by centrifugation
2. Cells were washed twice with cold PBS.
3. The supernatant was removed and discarded and the cell pellet was collected (At this point the cell pellet can be frozen at  $-80^{\circ}\text{C}$  and lysed at a later date).
4. The cell pellet was lysed in the appropriate extraction buffer for 30 minutes on ice with vortexing at 10 minute intervals.
5. Extracts were transferred to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at  $4^{\circ}\text{C}$ .
6. The clear lysate was aliquoted to clean microfuge tubes of 2 ml and realize a second tube of 20  $\mu\text{L}$ . These samples are ready for assay. Lysates can be stored at  $-80^{\circ}\text{C}$ .

***Sample pre-treatment:***

Serum was removed from the clot or cells as soon as possible after clotting and separation. Samples were aliquoted and stored frozen at  $20^{\circ}\text{C}$  to avoid loss of bioactive

Sample Treatment Buffer: When cells are lysed with Cell Extraction Buffer, incubate each sample and control with an equal volume of Sample Treatment Buffer on ice for 20 minutes.

***Standard preparation***

1. Diluted standards were serially Prepare immediately prior to use.

2. A 2,000 pg/mL Stock Standard was prepared by reconstituting one vial of the Human standard with distilled water
3. It was held at room temperature for 10-30 minutes. Add 225  $\mu$ L Sample diluent into all tubes.
4. A 1,000 pg/mL Standard 1 was prepared by adding 225  $\mu$ L of the 2,000 pg/mL Stock Standard to 225  $\mu$ L sample diluent to tube 1. Mix thoroughly and gently.
5. Standard 2 prepared by transferring 225  $\mu$ L from Standard 1 to tube 2. Mix thoroughly and gently.
6. Standard 3 was prepared by transferring 225  $\mu$ L from Standard 2 to tube 3. Mix thoroughly and gently and repeat the same for the 7 standard tubes.

***Assay Method***

1. Add 100  $\mu$ L of each standard to the appropriate standard wells (including the no standard blank control).
2. Add 50  $\mu$ L of 1X Sample Diluent to all the sample wells.
3. Add 50  $\mu$ L of each sample in duplicate to the sample wells.
4. Cover with adhesive film and incubate at room temperature (18° to 25°C) for 2 hours (microplate can be incubated on a shaker set at 400 rpm).
5. Wash microplate strips 6 times then add 100  $\mu$ L of BiotinConjugated Antibody to all wells and incubate at room temperature (18° to 25°C) for 1 hour

6. Wash microplate strips 6 times then add 100  $\mu$ L of StreptavidinHRP to all wells and incubate at room temperature (18° to 25°C) for 1 hour
7. Wash microplate strips 6 times and add 100  $\mu$ L of TMB Substrate Solution to all wells and. incubate the microplate strips at room temperature (18 to 25°C) for 30 minutes. Avoid direct exposure to intense light.
8. Stop the enzyme reaction by adding 100  $\mu$ L of Stop Solution into each well.
9. Read absorbance of each microplate on a spectrophotometer using 450 nm

## **VII. Radiological effects**

### ***Antitumor Effect – Solid Tumors***

Tumors were measured on CT and/or MRI scans every 8 weeks. Response and progression were evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)<sup>160</sup> Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

### ***Methods for Evaluation of Measurable Disease***

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4

weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imagingbased evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

### **VIII. Statistical analyses**

We evaluated the impacts of different administration schedules on drug exposures and adverse events of all grades. Correlation analyses were performed with logistic regression tests. Simulations were based on data from 26 patients. Simulation results for the regression coefficients for each variable in every group were compared to the results of the model fit to the original sample, to determine biases, precisions, and significances.

All statistical analyses were performed with a two-sided 0.05 alpha risk.

### **IX. Trial feasibility**

A Data safety and monitoring board (DSMB) meeting was planned after enrollment of 6 assessable patients in the 4 trial arms to assess the feasibility of the trial, based on preliminary toxicity and PK data. Here, we present the outcomes of this analysis.

### **X. Financial & competing interests disclosure**

Supported by Hospices Civils de Lyon (bourse Actions Incitatives); Ligue contre le Cancer; Association de Recherche contre le Cancer; Novartis SAS; Institut National du Cancer; and Roche (Recherche réalisé avec le soutien de Roche).

## **XI. Ethical conduct of research**

An appropriate institutional review board approval was obtained or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved. The EudraCT number of the study: 2012-002818-39

### **Methodology of data Analysis of the literature review**

The research was designed with 3 main consecutive steps: 1) to identify all early phase trials defining OBD for molecular targeted therapies; 2) to track all subsequent phases II and III trials, in which these defined OBD were tested; 3) to compare the OBD defined in early phases of drug development with the clinically effective doses, eventually approved by FDA.

1) *Identification of study drugs*: The search was performed on MEDLINE via PubMed (<http://www.pubmed.gov>), Google Scholar (<http://www.google scholar.com>), and [Clinicaltrials.gov](http://www.clinicaltrials.gov) (<http://www.clinicaltrials.gov>) to capture all English publications published between 2000 and 2016. In addition, relevant meeting abstracts from the American Society of Clinical Oncology (ASCO) were searched through ASCO website <http://meetinglibrary.asco.org/abstracts>. The search terms were « cancer »; « biological dose »; «targeted therapy »; «phase 1 trial» in the title or in the abstract. The studied compounds were classified into two major categories based on their chemical structures: 1) small inhibitor molecules; or 2) monoclonal antibodies. When molecular targeted therapies with reported OBD were identified in phase I trials (as a

primary endpoint or not), the web search was extended to compile the publications of subsequent phases II and III clinical trials testing these compounds. This was done by using the following keywords “compound name”, “dose”, “phase II trial” or “phase III trial”. Trials with more than one investigational molecular targeted agent were excluded.

- 2) *Comparison of OBD and clinically effective doses*: We assessed differences between the OBD defined in early phase trials, and the eventual clinical effective doses. Clinical effective doses were defined as the doses approved by FDA, if any; or the doses associated with positive outcomes in randomized phase III trials based on the primary endpoint. A “positive” trial was defined when the experimental arm was deemed superior to the standard arm by authors in superiority trials, not inferior in non-inferiority trials, or equivalent in equivalence trials, based on the trial primary objective.

## Appendix

### Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but	80	Normal activity with effort; some signs or symptoms of disease.

	ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Specific Guidance for Dose Reductions and Modifications based on Adverse Events

**Table (6): Specific dose modifications for hematologic adverse events (for within a cycle or at the beginning of a cycle)**

Adverse event	NCI CTCAE v.4 grade	Treatment administration	Dose modification
Neutropenia	Grade 1 and 2	Everolimus and sorafenib can be administered	No change
	Grade 3 or Grade 4 < 7 days	Delay administration until recovery to ANC $\geq$ 1,000/mcL	First occurrence: Hold everolimus and sorafenib until recovery to ANC $\geq$ 1,000/mcL. May then resume everolimus and sorafenib at full dose or decrease dose by one level  Second occurrence: Hold everolimus and sorafenib until recovery to ANC $\geq$ 1,000/mcL. May then resume everolimus and sorafenib and decrease dose by one level
	Grade 4 $\geq$ 7 days	Delay administration until recovery to ANC $\geq$ 1,000/mcL (grade 2)	Reduce dose by one level



<b>Febrile neutropenia</b>	≥ Grade 3	Delay administration until recovery to ANC ≥ 1,000/mcL (grade 2) and temperature ≤ 38 ° C	Reduce dose by one level
<b>Thrombocytopenia</b>	Grade 1	everolimus and sorafenib can be administered	No change
	Grade 2 and 3	Delay administration until recovery to platelets ≥ 75,000/mcL (grade 1)	First occurrence: Hold everolimus and sorafenib until recovery to platelets ≥ 75,000/mcL. May then resume everolimus and sorafenib at full dose or decrease dose by one level  Second occurrence: Hold everolimus and sorafenib until recovery to platelets ≥ 75,000/mcL. May then resume everolimus and sorafenib and decrease dose by one level
	Grade 4	Delay administration until recovery to platelets ≥ 75,000/mcL (grade 1) Manage with platelet transfusions if required	Reduce dose by one level
<b>Thrombocytopenic bleeding</b>	Not in CTCAE v4 but defined as platelets < 50 x 10 <sup>9</sup> /L and associated with clinically significant bleeding	Delay administration until recovery to platelets ≥ 75,000/mcL (grade 1) Manage with platelet transfusions if required	Reduce dose by one level

**Table (7): Dose modifications for non-hematological toxicities.**

<b>Adverse event</b>	<b>NCI CTCAE v.4 grade</b>	<b>Everolimus and sorafenib administration</b>
Non-hematological toxicity (except diarrhea)	Grade 1	No change
	Grade 2 toxicity	Hold everolimus and sorafenib till resolution or amelioration of AE to tolerable grade 1 or better. May then resume everolimus and sorafenib at full dose or decrease dose by one level
	Grade 3	Hold everolimus and sorafenib till resolution or amelioration of AE to grade 1 or better. May then resume everolimus and sorafenib and decrease dose by one more dose level
	Grade 4	Off study



**Table (8): Specific dose modifications for diarrhea.**

<b>Diarrhea</b>		
<b>NCI CTCAE v.4 grade</b>	<b>Management</b>	<b>Everolimus and sorafenib administration</b>
<b>Grade 1 for more than 24 hours</b>	<ul style="list-style-type: none"> <li>- Research of other/concomitant causes of diarrhea*</li> <li>- Dietary advice**</li> <li>- Loperamide 4 mg followed by 2 mg q4h or after each unformed stool (maximum 16 mg/day), continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- Racecadotril 100 mg q8h</li> </ul>	No change
<b>Grade 2</b>	<ul style="list-style-type: none"> <li>- Research of other/concomitant causes of diarrhea*</li> <li>- Dietary advice**</li> <li>- Loperamide 4 mg followed by 2 mg q4h or after each unformed stool (maximum 16 mg/day), continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- Racecadotril 100 mg q8h continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- May try diosmectite, 1 dose at each meal continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> </ul>	Hold everolimus and sorafenib till resolution or amelioration of AE to grade 1 or better. May then resume everolimus and sorafenib at full dose or decrease dose by one level
<b>Grade 2 persisting ≥ 24 h despite above treatment or Grade 3</b>	<ul style="list-style-type: none"> <li>- Consider hospitalization for supportive care including hydration</li> <li>- Research of other/concomitant causes of diarrhea*</li> <li>- Dietary advice**</li> <li>- Loperamide (4 mg followed by 2 mg q4h or after each unformed stool (maximum 16 mg/day), continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- Racecadotril 100 mg q8h continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- If ineffective, may try diosmectite, 1 dose at each meal continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- If ineffective, may add octreotide 150 mcg SC tid, to be continued until 24 hours after the end of diarrhea</li> </ul>	Hold everolimus and sorafenib till resolution or amelioration of AE to grade 1 or better. May then resume everolimus and sorafenib and decrease dose by one level

<b>Grade 4</b>	<ul style="list-style-type: none"> <li>- Hospitalization for supportive care including hydration</li> <li>- Research of other/concomitant causes of diarrhea* - Dietary advice**</li> <li>- Loperamide 4 mg followed by 2 mg q4h or after each unformed stool (maximum 16 mg/day), continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- Racecadotril 100 mg q8h continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- If ineffective, may try diosmectite, 1 dose at each meal continued for 12 hours following resolution of the diarrhea and reestablishment of a normal diet</li> <li>- If ineffective, may add octreotide 150 mcg SC tid, to be continued until 24 hours after the end of diarrhea</li> <li>- If ineffective, may add octreotide 150 mcg SC tid, to be continued until 24 hours after the end of diarrhea</li> </ul>	Off study
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**Table (9): Specific dose modifications for hand-foot syndrome.**

<b>Hand foot syndrome</b>		
NCI CTCAE v.4 grade	Management	Everolimus and sorafenib administration
<b>Grade 1 for more than 24 hours</b>	<ul style="list-style-type: none"> <li>- Preventive measures**</li> <li>- Patients should avoid hot water and should use moisturizing creams for relief.</li> <li>- Keratolytics for hyperkeratotic lesions, such as urea 20%–40%, or salicylic acid 6% twice daily may be indicated.</li> <li>- Cotton gloves and socks can be worn at night to prevent further injury and to help retain moisture.</li> </ul>	No change
<b>Grade 2</b>	<ul style="list-style-type: none"> <li>- The same as for grade 1 toxicity.</li> <li>- Consider applying clobetasol 0.05% or fluocinonide 0.05% ointment to erythematous areas twice daily.</li> <li>- For pain control, consider using topical analgesics such as lidocaine 2%</li> </ul>	1 <sup>st</sup> occurrence: Hold everolimus and sorafenib till resolution or amelioration of AE to grade 1 or better. May then resume everolimus and sorafenib at full dose or decrease dose by one level *. 2 <sup>nd</sup> , 3 <sup>rd</sup> and 4 <sup>th</sup> occurrence: Interrupt treatment for a minimum of 7 days until the HFS reaches grade 1 or 0, and then resume treatment at the lower level dose *. 5 <sup>th</sup> occurrence: Stop of treatment. Removal of the study.
<b>Grade 3</b>	<ul style="list-style-type: none"> <li>- The same as for grade 1 toxicity.</li> <li>- Consider applying clobetasol 0.05% or fluocinonide 0.05% ointment to erythematous areas twice daily.</li> <li>- For pain control, consider using topical analgesics such as lidocaine 2%</li> </ul>	1 <sup>st</sup> and 2 <sup>nd</sup> and 3 <sup>rd</sup> occurrence: Hold everolimus and sorafenib till resolution or amelioration of AE to grade 1 or better. Then resume everolimus and sorafenib and decrease dose by one level*.

		4 <sup>th</sup> occurrence: Stop of treatment. Removal of the study.
<b>Grade 4</b>	<ul style="list-style-type: none"> <li>- The same as for grade 1 toxicity.</li> <li>- Consider applying clobetasol 0.05% ointment to erythematous areas twice daily.</li> <li>- For pain control, consider using topical analgesics such as lidocaine 2%</li> </ul>	Off study

**Table (10): Specific dose modifications for non infectious pneumonitis.**

<b>Non infectious pneumonitis</b>		
<b>NCI CTCAE v.4 grade</b>	<b>Management</b>	<b>Everolimus and sorafenib administration</b>
<b>Grade 1: asymptomatic</b>	No specific measure	No change
<b>Grade 2: symptomatic but not interfering with activities in daily living</b>	Consider corticosteroid therapy.	Decrease everolimus dose to lower dose level until grade 1 or lower Hold everolimus if symptoms are troublesome Discontinue everolimus if no recovery to grade 1 occurs within 3 weeks
<b>Grade 3: symptomatic interfering with activities in daily living</b>	Consider corticosteroid therapy.	Hold everolimus until recovery to grade 1 or less and restart within 2 weeks at reduced dose (lower dose level)
<b>Grade 4: Life threatening</b>	Consider corticosteroid therapy.	Discontinue everolimus

## **Response Criteria**

### **Evaluation of Target Lesions**

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

### **Evaluation of safety parameters**

Adverse events (AE) defined as any untoward medical occurrence or/and unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Serious adverse events (SAE) defined as any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (the term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires in-patient hospitalization or prolongation of existing hospitalization

- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a medically significant event:

The intensities of all adverse events were graded with the Common Terminology Criteria for Adverse Events (CTCAE) grading system v4.0, according to the corresponding toxicity categories and actions were taken regarding study medication either by treatment discontinuation or dose modification following Specific Guidance for Dose Reductions and Modifications based on Adverse Events (as described in Appendix). All adverse events (AE) regardless of seriousness or relationship to Investigational Product that occurred after the informed consent up to 60 days after the last study drug administration were recorded in the AE pages of the CRF.

## Results

### **I. Patient Characteristics**

Twenty nine patients were enrolled between May 2013 and December 2015, but 3 were screen failures due to early progression before the start of treatment. Consequently, 26 patients received minimum 1 dose of either drug. The distribution of patients by arms, along with patient characteristics are presented in Table (11) A high proportions of patients with cholangiocarcinoma (30.8%) has been enrolled. Indeed because very favorable outcomes had been observed for the first 2 patients, many patients of the region with this disease have been referred to our center for inclusion in EVESOR trial.

Patients had different solid tumor types, including: cholangiocarcinoma (n=8), colon-rectum adenocarcinoma (n=5), breast cancer (n=3), pancreatic adenocarcinoma (n=3), cervical cancer (n=1), lung cancer (n=1), ovarian cancer (n=1), hepatocellular carcinoma (n=1), fallopian tube adenocarcinoma (n=1), anus squamous cell carcinoma (n=1), and endometrial adenocarcinoma (n=1).

As per protocol, patients were consecutively assigned to the 4 different treatment arms, as shown in Table 11. In schedules A and B, all patients were treated on dose level 1. In schedule C, 6 patients were treated on dose level 1. In schedule D, 3 patients were treated on dose level 1, while 3 more patients were treated on dose level 2.



**Table (11): Patient demographics and clinical characteristics**

<b>Characteristics</b>	<b>No. of patients (N=26)</b>	<b>%</b>
<b>Sex</b>		
Male	15	57.7
Female	11	42.31
<b>Age, years</b>		
Median	62.5	
Range	34-73	
<b>Arm A (n=7, dose level 1):</b>		
Colon rectum adenocarcinoma	2	28.6 14.3
Breast cancer	1	14.3 28.6
Pancreatic adenocarcinoma	1	14.3
Cholangiocarcinoma	2	
Hepatocellular carcinoma	1	
<b>Arm B (n=7, dose level 1)</b>		
Colon-rectum adenocarcinoma	1	14.3 14.3
Fallopian tube Adenocarcinoma	1	42.8 14.3
Cholangiocarcinoma	3	14.3
Lung cancer	1	
Breast cancer	1	
<b>Arm C (n=6, dose level 1)</b>		
Endometrial adenocarcinoma	2	33.3 33.3
Cholangiocarcinoma	2	16.6
Breast cancer	1	16.6
Colon rectum adenocarcinoma	1	
<b>Arm D (n=6, dose level 1, n=3 ; dose level 2, n=3))</b>		
Colon-rectum adenocarcinoma	1	16.6 16.6
Colon-rectum adenocarcinoma	1	16.6 16.6
Cervix cancer	1	16.6
Fallopian tube adenocarcinoma	1	16.6
Pancreatic adenocarcinoma	1	
Anus squamous cell carcinoma	1	
Cholangiocarcinoma		

<b>Prior Treatments</b>		
1-2	4	15.4
$\geq 3$	22	84.6

## II. Dose limiting toxicities

During dose escalation in administration *schedule C* we observed dose limiting toxicities in two patients (n=2) within the first twenty-eight days of treatment at dose level 1. One patient (n=1), patient 17 who experienced liver abscess grade 3 at Cycle 1 Day 15, while the second patient (n=1), patient 23 at Cycle 1 Day 8 experienced toxedemia grade 3. Both toxicities were considered related to study treatment and resolved at treatment discontinuation. Thirteen enrolled patients died. The causes of death were disease progression in 12 patients and lung infection in a patient on Arm B Cycle 2 Day 15, potentially related to study treatment.

## III. Safety and Tolerability

The combination of the two drugs was generally well tolerated in all four administration schedules (Table 12). All patients experienced at least one adverse event, but the severities of these toxicities were mainly grades 1 and 2. The most frequent treatment-related clinical adverse events among all grades included fatigue (69.2%), skin rash (38.5%), anorexia (30.8%), hand foot syndrome (30.8%), constipation (26.9%) and stomatitis (23.1%). The most frequent treatment-related biological adverse events among all grades included hypophosphatemia (23.1%) and anemia (19.23%). The most frequent treatment-related adverse events of grade 3 severity included fatigue (23.1%), infections (7.7%), and hypophosphatemia (11.5%). Only one grade 4 treatment-related adverse event was observed: a hepatic abscess

that was developed 10 days after the second liver biopsy, in a patient in Arm A, Cycle 2 day 6.

*Fatigue* (69.2 %), was the most frequent adverse event. The severity of fatigue was ranging from grade 1 to 2 as well as grade 3 to 4 for some patients. It was resolved within 1-6 weeks and it was assessed to be definitely related to treatment drugs for most of the patients.

*Skin rash* (38.5%), was the second most frequent adverse events in particular rash or dermatitis acneiform, which was mainly on the face, scalp, chest and back. Occurrence of treatment related rash and dermatitis of grade 1 to 2 was proportional with continuous and intermittent treatment schedules, arising less frequently in schedules C and D. Skin dryness, prurities and allergic reactions were also seen. No events of squamous cell carcinoma or other proliferative skin lesions were recorded. The onset happened within 4-6 weeks from starting treatment drugs and it was resolved within 7 to 10 days.

*Anorexia (30.8%) and hand foot syndrome (30.8%)*, The severity of anorexia as well as hand and foot syndrome was ranging from grade 1 to 2 and no grade 3 or 4. The occurrence of both adverse events were higher in continuous schedule A and B (57.1%, 28.6%) as compared to intermittent schedules C (16.6%, 28.8%) and D (20% and 33.3%) for anorexia and hand and foot syndrome respectively.

### **Less frequent treatment related adverse events :**

- **Constipation**

The frequency of constipation was 21.7% mainly grade 1 to 2 in severity except for one patient who experienced grade 3 to 4 toxicity.

Patients are given either lactulose or macrogol for one week until resolution of the constipation

- **Decreased number of blood cells.**

Thrombocytopenia all grades was experienced by 15.4% of patients on study. The severity was mainly of grade 1 to 2 except for one patient who experienced thrombocytopenia grade 3 to 4. Their platelets were monitored as per study calendar (patients and methods, appendix). No dose reductions or modifications of either drugs were needed.

Thrombocytopenia was resolved within 3 to 4 weeks.

- **Nausea/vomiting.**

The frequency of nausea and vomiting all grades were 11.5% and 17.4% respectively. Patients were treated initially with metoclopramide 10 mg q8h po. The onset of the adverse event is within 4-8 weeks and it's rapidly manageable within few days.

- **Stomatitis**

The frequency of stomatitis experienced by all patients was 23.1% that was lower than expected as stomatitis has been observed in 40% patients treated with everolimus. It is usually mild at lower dose and the incidence decreases with subsequent cycles. Patients have been recommended to use fungizone (bain douche) three times daily. <sup>161</sup>.

- **Diarrhea.**

The frequency of diarrhea was 23.1% with severity of grade 1 to 2. Prophylactic anti-diarrheal medications are not suggested for therapy for diarrhea that occurs during treatment with everolimus and sorafenib, following specific guidelines for dose reductions and modifications

(patients and methods, appendix), stating that no dose reduction is needed for these patients.

- **Electrolyte abnormalities.**

Hypophosphatemia was treated by giving patients oral sodium acid phosphate solution (phosphoneuros) 3 to 4 times a day. While Hypocalcemia and hypokalemia were treated with Cacit 1000 mg once per day and potassium chloride capsules twice a day (Diffu K). The severity of electrolyte imbalance was varying between grade 1 to 2: hypophosphatemia (23.1%), hypocalcemia (11.5%), hypokalemia (11.5%) and hypocalcemia (3.8%). The study drugs have been interrupted for two patients experienced hypophosphatemia. The duration of hypophosphatemia AE is varying between one week and 10 days, while the duration of hypocalcemia and hypokalemia was varying between 3 and 5 days.

- **High blood pressure**

High blood pressure was experienced by a frequency of 7.7% mainly grade 1 to 2 in severity. Angiotensin receptor blocker and ACE inhibitors were given to manage the symptoms of hypertension.

To summarize ; Schedule A, the most frequent treatment related adverse events were fatigue (85.7%) followed by anorexia (57.1%), hand foot syndrome (57.1%), skin rash (42.8%), diarrhea (42.8%). and stomatitis (42.8%). Three patients needed dose reduction of either everolimus (n=2/7) to 2,5 mg at C2D1 or sorafenib (n=1/7) to 200 mg at C2D6 which was mainly due to recurrent hypophosphatemia adverse event.

On the other hand, fatigue and skin rash (57.1%), constipation (42.8%) were the most common drug-related adverse events, predominantly of grade 1 and 2 arose on schedule B. Two patients in this schedule needed dose reduction of sorafenib to 200 mg during the first cycle of treatment in C1D11 and C1D15, before the addition of everolimus.

Treatment related adverse events were less frequently observed in treatment schedules C and D. The most commonly recorded AE in schedule C were fatigue grade 1 to 2 (66.6%) and grade 3 to 4 (16.6%); and skin rash grade 1 to 2 (50%) and grade 3 to 4 (16.6%). While in schedule D nausea grade 1 to 2 (n=50%), fatigue grade 1 to 2 (66.6%) and grade 3 to 4 (33.3%) were the most common observed toxicities Only one patient in each administration schedule C and D needed dose reductions of sorafenib to 200 mg during C1D15 and C1D22 respectively.

*Most commonly experienced adverse events Potentially Related to Everolimus and/or sorafenib and their managements*

Table (12): Treatment related adverse events

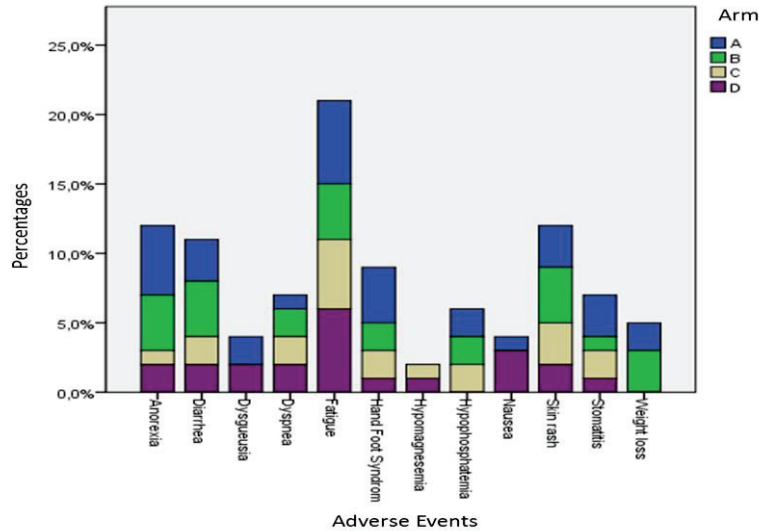
Administration Schedule	Continuous schedules				Intermittent schedules				All schedules	
	Arm A: n=7		Arm B: n=7		Arm C: n=6		Arm D: n=6		Total n=26	
	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4
<b>Biological AE</b>										
Hypokalemia	2(28.6%)	1(14.3%)	0(0%)	1(14.3%)	0(0%)	0(0%)	0(0%)	0(0%)	3(11.5%)	2(8.7%)
Thrombocytopenia	3(42.8%)	0(0%)	0(0%)	0(0%)	1(16.6%)	1(16.6%)	0(0%)	0(0%)	4(15.4%)	1(3.8%)
Hypophosphphatemia	2(28.6%)	1(14.3%)	2(28.6%)	1(14.3%)	2(33.3%)	1(16.6%)	0(0%)	0(0%)	6(23.1%)	3(11.5%)
Lymphopenia	1(14.3%)	1(14.3%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	1(3.8%)
Hypocalcemia	0(0%)	0(0%)	3(42.8%)	1(14.3%)	0(0%)	0(0%)	0(0%)	0(0%)	3(11.5%)	1(3.8%)
Liver transaminase increase	1(14.3%)	1(14.3%)	0(0%)	0(0%)	1(16.6%)	1(16.6%)	0(0%)	0(0%)	1(3.8%)	1(3.8%)
Anemia	0(0%)	0(0%)	2(28.6%)	1(14.2%)	1(16.6%)	0(0%)	2(33.3%)	0(0%)	5(19.23%)	2(8.7%)
Hemolysis	0(0%)	0(0%)	1(14.3%)	1(14.3%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	1(3.8%)
Low albumin	0(0%)	0(0%)	0(0%)	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	1(4.3%)	0(0%)
Cholestasis	0(0%)	0(0%)	2(28.6%)	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	2(7.7%)	1(3.8%)
<b>Clinical AE</b>										
High Blood Pressure	1(14.3%)	0(0%)	1(14.3%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	2(7.7%)	0(0%)
Pain	1(14.3%)	0(0%)	2(28.6%)	0(0%)	0(0%)	0(0%)	3(50%)	0(0%)	6(23.1%)	0(0%)
Purpura	0(0%)	0(0%)	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)
Oedema	1(14.3%)	1(14.3%)	0(0%)	0(0%)	1(16.6%)	0(0%)	0(0%)	0(0%)	3(11.5%)	1(3.8%)
Hot flush	0(0%)	0(0%)	0(0%)	0(0%)	1(16.6%)	0(0%)	0(0%)	0(0%)	2(7.7%)	0(0%)
Infection	0(0%)	0(0%)	0(0%)	0(0%)	2(33.3%)	0(0%)	0(0%)		2(7.7%)	2(7.7%)
Oncolysis	1(14.2%)	0(0%)	0(0%)	0(0%)	1(16.6%)	0(0%)	0(0%)	0(0%)	2(7.7%)	0(0%)
Tachycardia	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)
Stomatitis	3(42.8%)	0(0%)	1(14.3%)	0(0%)	1(16.6%)	0(0%)	1(16.6%)	0(0%)	6(23.1%)	0(0%)

<b>Pruritis</b>	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)
<b>Alopecia</b>	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)
<b>Dysphonia</b>	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)
<b>Fever</b>	0(0%)	0(0%)	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)
<b>Gastritis</b>	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(20%)	0(0%)	1(3.8%)	0(0%)
<b>Nausea</b>	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	3(50%)	0(0%)	3(11.5%)	0(0%)
<b>Anorexia</b>	4(57.1%)	0(0%)	2(28.6%)	0(0%)	1(16.6%)	0(0%)	1(20%)	0(0%)	8(30.8%)	0(0%)
<b>Skin rash</b>	3(42.8%)	0(0%)	4(57.1%)	0(0%)	3(50%)	1(16.6%)	0(0%)	0(0%)	10(38.5%)	1(3.8%)
<b>Dysgeusia</b>	2(28.6%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	2(33.3%)	0(0%)	4(15.4%)	0(0%)
<b>Weightloss</b>	2(28.6%)	0(0%)	2(28.6%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	4(15.4%)	0(0%)
<b>Hand foot syndrome</b>	4(57.1%)	0(0%)	2(28.6%)	0(0%)	2(28.6%)	0(0%)	2(33.3%)	0(0%)	8(30.8%)	0(0%)
<b>Constipation</b>	2(28.6%)	0(0%)	3(42.8%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	5(21.7%)	0(0%)
<b>Diarrhea</b>	3(42.8%)	0(0%)	2(28.6%)	0(0%)	1(20%)	0(0%)	1(20%)	0(0%)	6(23.1%)	0(0%)
<b>Allergy</b>	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)
<b>Fatigue</b>	6(85.7%)	1(14.2%)	4(57.1%)	2(33.3%)	4(66.6%)	1(16.6%)	4(66.6%)	2(33.3%)	18(69.2%)	6(23%)
<b>Interstitial pneumopathy</b>	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(20%)	1(20%)	1(3.8%)	1(3.8%)
<b>Thrombotic Microangiopathy</b>	0(0%)	0(0%)	1(14.2%)	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	1(3.8%)
<b>Dyspnea</b>	1(14.2%)	0(0%)	2(28.6%)	0(0%)	2(28.6%)	0(0%)	1(20%)	0(0%)	7(11.5%)	0(0%)
<b>Alopecia</b>	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)
<b>Bleeding</b>	0(0%)	0(0%)	1(14.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(3.8%)	0(0%)

The risk of treatment-related adverse events was higher in continuous schedules A and B than in intermittent schedules C and D (64.1% vs. 35.9%,  $P < 0.0001$ ). Compared to intermittent arms, continuous arms were associated with higher risks of anorexia (52.9% vs 25.0%), diarrhea (50% vs 33%), and hand foot syndrome (42.8% vs 16.6%).



However, risks of fatigue (71.4% vs 83.3%) and skin rash (50.0% vs 41.6%) were similar between continuous and intermittent arms (Figure 7).



**Figure (7):** Frequency of adverse events in different treatment schedules A, B, C and D.

#### IV. Serious Adverse Event (SAEs)

Data Safety and Monitoring board (DSMB) meeting aimed to review accumulating safety and tolerability data generated in the study. Based on the last communicated report covering a 2-year period for 16 patients included a total number of nine (n=9) patients experienced 15 serious adverse events during a total of 35 cycles that were reported to the sponsor. The overall 15 SAEs occurred after inclusion in the four administration schedules A, B, C and D but 3 SAEs occurred between inclusion date and before the first date of treatment. Among the 15 SAEs, 3 fatal cases in arm

A (2 cases, 28.5%) and in arm C (1 case, 20%) were reported. Among the 15 SAEs, 7 cases were related to the IMPs which were assessed as being expected reactions. 11 cases required hospitalization in arm A (4 cases, 57.1%), in arm B (5 cases, 71.4%), in arm C (1 case, 20%) and in arm D (1 case, 25%) and 1 case in arm A (1 case, 14.2%) was life threatening. The highest number of serious adverse event was reported in arm A as 100% of patients experienced SAEs while only one SAE was reported in arm D. The seriousness criteria and the outcome of the serious adverse events are described in the table (13) below.

**Table (13): Criteria of gravity of serious adverse events**

Criteria of gravity	Number of SAE reports							
	Arm A: 7		Arm B:7		Arm C:5		Arm D:4	
	N	%	N	%	N	%	N	%
<b>Fatal</b>	2	28.5%	0	0	1	20%	0	0
<b>Life threatening</b>	1	14.2%	0	0	0	0	0	0
<b>Hospitalisation</b>	4	57.1%	5	71.4%	1	20%	1	25%
<b>Persistent disability/incapacity</b>	0	0	0	0	0	0	0	0
<b>Birth defect/congenital anomaly</b>	0	0	0	0	0	0	0	0
<b>Medically important condition</b>	NA	NA	NA	NA	NA	NA	NA	NA
<b>Total</b>	7	100%	5	71.42%	2	40%	1	25%

Among the 15 serious adverse events, 7 were related to the investigational medicinal products (IMP) (everolimus and/or sorafenib) which were: pneumonia, pain and hypophosphoremia

*Schedule A:* 3 out of 7 SAEs were deemed to be related to IMP and described as pneumonia (n=1); reduced blood phosphorous (n=1) and general pain (n=1)

*Schedule B:* 3 out of 6 SAEs were deemed to be related to IMP and described observed diarrhea (n=1), vomiting (n=1), general physical health deterioration (n=1) and hemolytic anemia (n=1).

*Schedule C:* A total of 4 SAEs were assessed as being unrelated to IMP and described as bile duct stenosis (n=1), general physical health deterioration (n=1) and disease progression (n=2)

*Schedule D:* pyrexia was the only SAE observed and described to be related to IMP

These 15 SAEs contained 18 reactions that were classified according to variable system organ class (SOC) and were reported in the four administration schedules as listed below in table 14

**Table (14): Analysis of serious adverse events by system organ class (SOC)**

System Organ Class (SOC)	Scheduled treatment	Scheduled treatment	Scheduled treatment	Scheduled treatment
<b><u>Infections and infestations</u></b>				
<b>Body system / ADR Term</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
Pneumonia	1			
<i>Subtotal</i>	1	0	0	0
<b><u>Respiratory, thoracic and mediastinal disorders</u></b>				
<b>Body system / ADR Term</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
Pulmonary embolism	1			
<i>Subtotal</i>	1	0	0	0
<b><u>Gastrointestinal disorders</u></b>				
<b>Body system / ADR Term</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
Abdominal pain	1			
Diarrhea		1		
Vomiting		1		
Rectal stenosis		1		
<i>Subtotal</i>	1	3	0	0
<b><u>Investigations</u></b>				
<b>Body system / ADR Term</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
Blood phosphorus decreased	1			
<i>Subtotal</i>	1	0	0	0
<b><u>General disorders and administration site conditions</u></b>				
<b>Body system / ADR Term</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
Pain	1			
Disease Progression	2		2	
Pyrexia				1
General physical health deterioration		1	1	
<i>Subtotal</i>	3	1	3	1

<b><u>Blood and lymphatic system disorders</u></b>				
<b>Body system / ADR Term</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
Haemolytic anaemia		1		
<i>Subtotal</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>
<b><u>Vascular disorders</u></b>				
<b>Body system / ADR Term</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
Cerebrovascular accident		1		
<i>Subtotal</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>
<b><u>Hepatobiliary disorders</u></b>				
<b>Body system / ADR Term</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
Bile duct stenosis			1	
<i>Subtotal</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>
<b>TOTAL</b>	<b>7</b>	<b>6</b>	<b>4</b>	<b>1</b>

### ***Suspected Unexpected Serious Adverse Reactions (SUSARs)***

Immuno-allergic events, *classified as Suspected, Unexpected Serious Adverse Reactions*, included a grade 3, negative Coombs test hemolysis in Arm C, Cycle 1 Day 15; a thrombotic micro-angiopathy in Arm B, Cycle 2 Day 1; and grade 2-3 toxidermias in Arm B, Cycle 1 Day 10 and Arm C, Cycle 1 Day 8, respectively. Moreover, serious liver infectious episodes were observed in the context of liver biopsies, including a grade 3 liver abscess in Arm C, Cycle 1 Day 15 (14 days after a liver biopsy); a grade 4 liver abscess in Arm A, Cycle 2 Day 6 (10 days after the second biopsy); and a grade 3 febrile cholestasis in Arm B, Cycle 2 Day 1 (1 month after a biopsy). As a result, it was subsequently decided to stop performing the second liver biopsy.

Following the specific guidance of dose reduction based on adverse events according to CTCAE v4.0, dose reductions or interruptions were reported in a total of 7 patients included in the study all of them being due to toxicities with an average occurrence of 9 adverse events.

## V. Pharmacokinetic models for sorafenib and everolimus

Sorafenib and everolimus PK were modeled independently. The structural model of sorafenib was a one compartment model with first order absorption (ADVAN2 TRANS2), while the structural model of everolimus was two compartment model with first order absorption (ADVAN4 TRANS4). No relevant graphical/statistical relationships were found between screened covariates such as sex, age, pathology, creatinine and unexplained variability of estimated parameters. In addition, there was a minimal change in objective functions after stepwise addition of each covariate to the structural model. For sorafenib, apparent oral clearance (Cl/F) was estimated to be 5.10E+00 L/h and central apparent volume of distribution (Vd/F) was estimated to be 2.31E+02 L. The inter-individual variability was 30.9% CV for apparent oral clearance, 75.7% CV for Vd and 75.7% CV for inter-occasion variability (IOV). The absorption rate constant (Ka) was 3.57E-01 /h (IIV 97.3%). For everolimus, apparent oral clearance (Cl/F) was 4.23E+00 L/h, central apparent volume of distribution (Vd<sub>central</sub>/F) was 1.52E+02 L, inter-compartment clearance (Q/F) was 6.72E+01 L/h, peripheral volume of distribution (V2/F) was 3.36E+02 L. Exponential Residual Error (SIGMA) is fixed to 1. The interindividual variability was 99.9 % for Cl, 60.5% for Vd and 66% for IOV. The absorption rate constant (Ka) was estimated at 0.005/h (IIV 5.8%).

The exponential residual error (SIGMA) was fixed to 1 to improve modeling convergence in the context of a large residual error.

**Table (15): Compartmental estimated PK parameters for sorafenib and everolimus.**

Parameter	Estimate	*RSE on Estimate (%)	**IIV (%CV)	***RSE on IIV (%)
<u>Sorafenib</u>				
CL/F,L/h	5.10E+00	(12.9%)	30.9%	29.0%
Vd <sub>central</sub> /F, L	2.31E+02	(21.3%)	75.7%	26.2%
Ka,1/h	3.57E-01	(36.1%)	97.3%	48.4%
SIGMA	1 FIX	NA	NA	NA
IOV	NA	NA	0.15 (38.6%)	24.6%
<u>Everolimus</u>				
CL/F, L/h	4.23E+00	(30.2%)	99.9 %	51.1%
Vd <sub>central</sub> /F, L	1.52E+02	(12.7%)	60.5%	31.6%
Q/F,L/h	6.72E+01	(23.2%)	54.2%	28.14%
V2 <sub>peripheral</sub> /F, L	3.36E+02	(24.4%)	73.9 %	26.02%
Ka,1/h	9.37E-01	(26.4%)	85.8%	53.5%
SIGMA	1 FIX		NA	NA
IOV	NA		0.435(66%)	59%

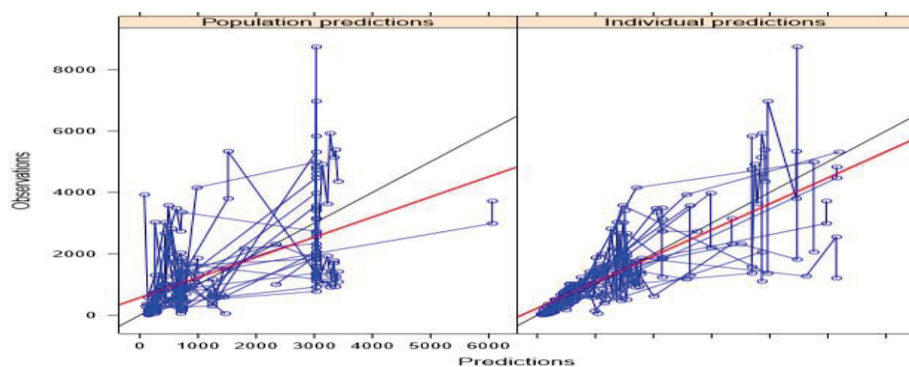
CL/F=apparent oral clearance; F=bioavailability; IOV=inter-occasion variability; ka=absorption rate constant; Q=inter-compartmental clearance; V1/F=volume of distribution of central compartment after oral administration; V2/F=volume of distribution of peripheral compartment after oral administration; SIGMA: model error for residual variability. \*RSE = Relative standard error % (Standard error / parameter) \* 100 . \*\* IIV = CV = Interindividual Variability=Coefficient of Variation= sqrt (var.rand.effect) \* 100 . \*\*\* RSE = Relative standard error % on standard deviation scale

## Qualification of the PK models

### Goodness of fit

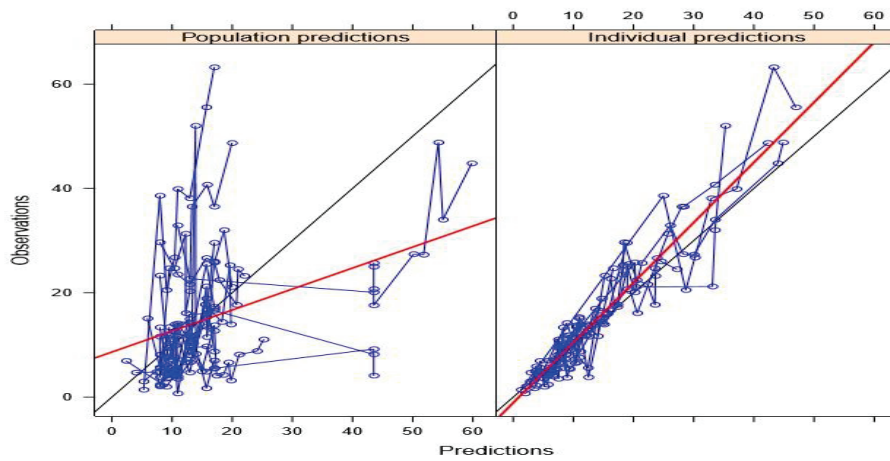
Goodness-of-fit-plots were generated for the two structural models (Figure 8 and Figure 9).

Individual predictions were consistent with observed values across the range of observations. Individual weighted residuals were uniformly distributed across the range of individual predictions, and weighted residuals were evenly scattered across time, after each dose (Figure 10 and Figure 11). The structural model performance evaluations of the two drugs were performed with visual predictive checks (VPCs). The VPCs revealed that the model-simulated predictions were in agreement with the observed concentrations that were used for structural model building. No apparent bias was observed (Figure 12 and Figure 13). A representative plot of few individuals is provided below (Figure 14 and Figure 15). This plot would give an overall trend of fitted concentrations. It could be seen that for certain individuals the population predictions are underpredicted or overpredicted.

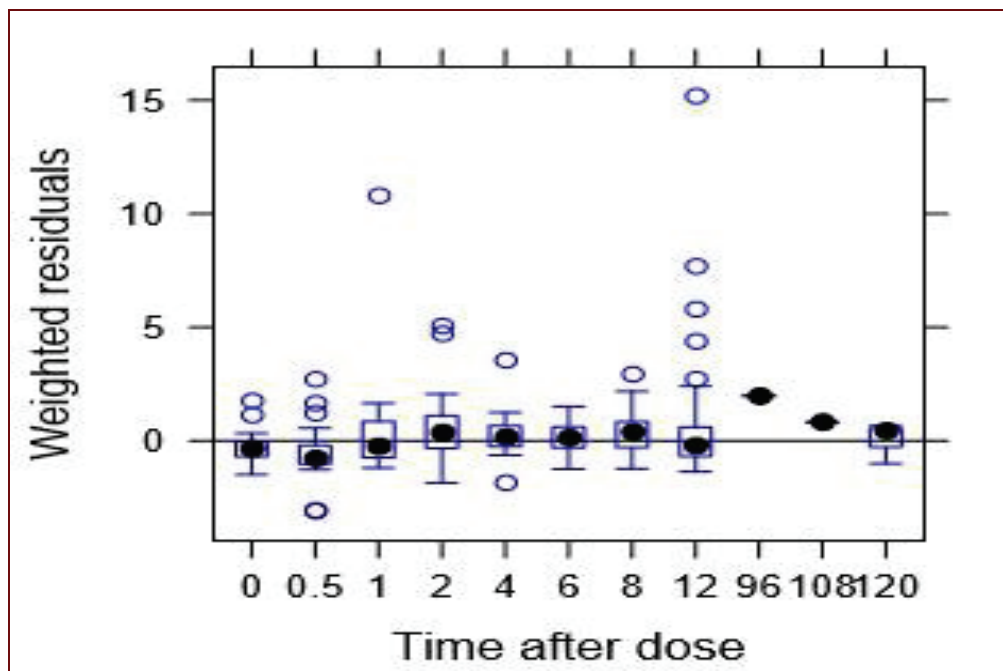


**Figure (8):** Diagnostic goodness-of-fit plots for the sorafenib structural model. Predicted versus observed concentrations are shown for (*left*) the population and for (*right*) individuals. Black line: identity line; Red line: smoothing of predictions (trend)

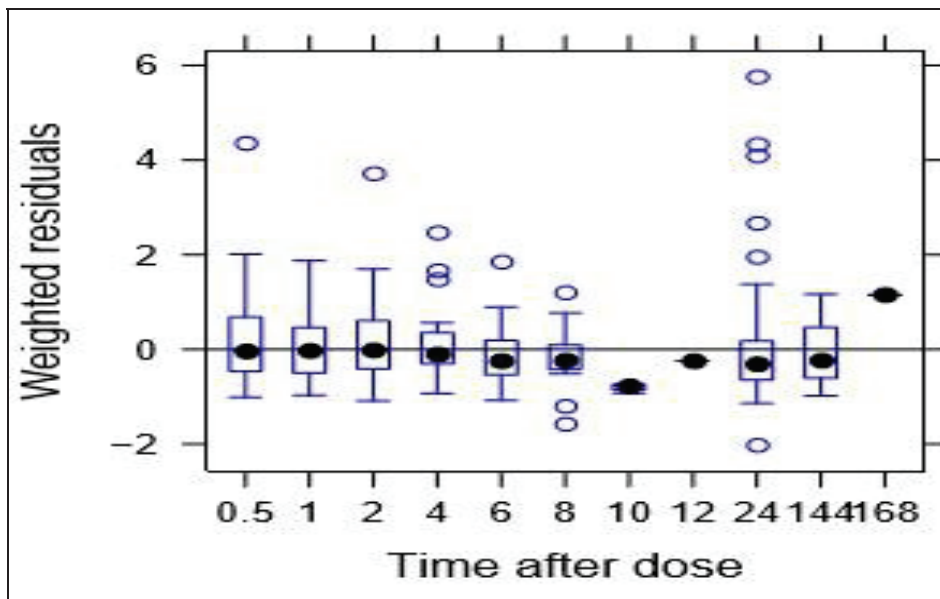




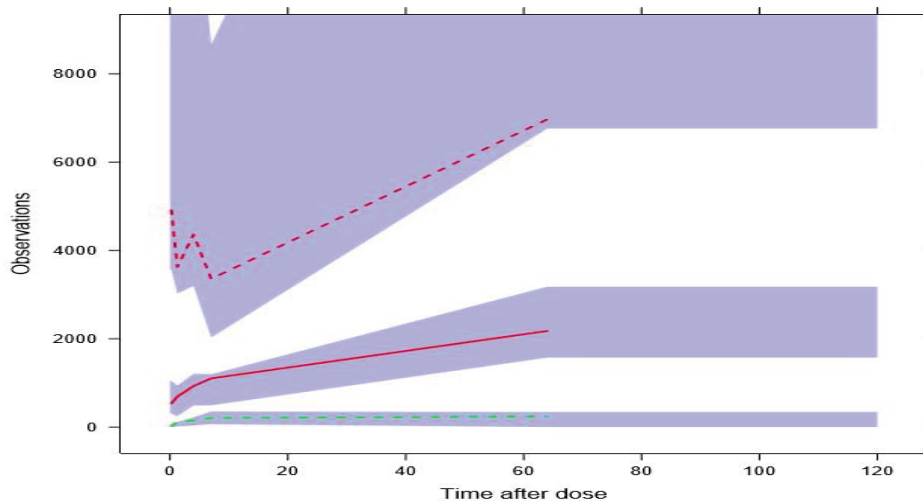
**Figure (9):** Diagnostic goodness-of-fit plots for the everolimus structural model. Predicted versus observed concentrations are shown for (*left*) the population and for (*right*) individuals. Black line: identity line; Red line: smoothing of predictions (trend).



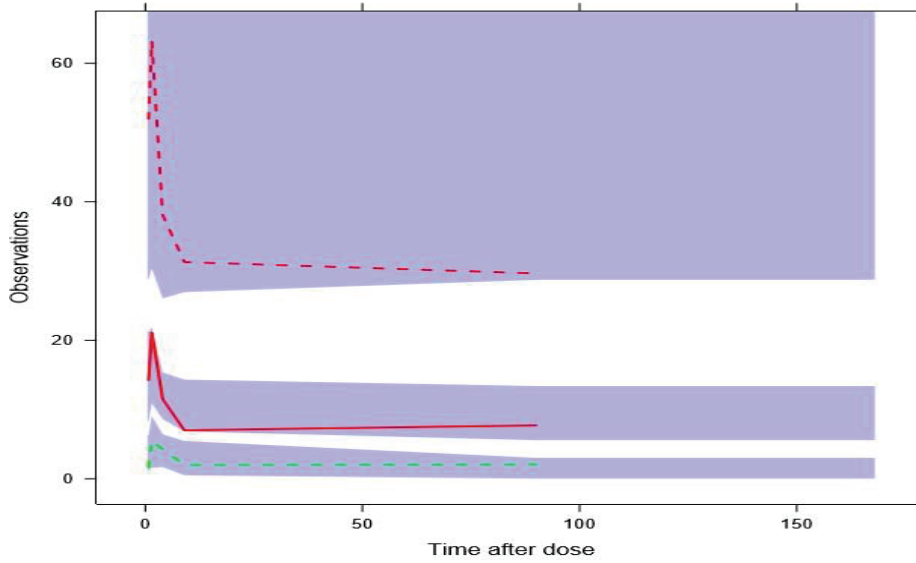
**Figure (10):** Diagnostic goodness-of-fit plots for the sorafenib structural models, showing weighted residuals versus time (hours) after dose.



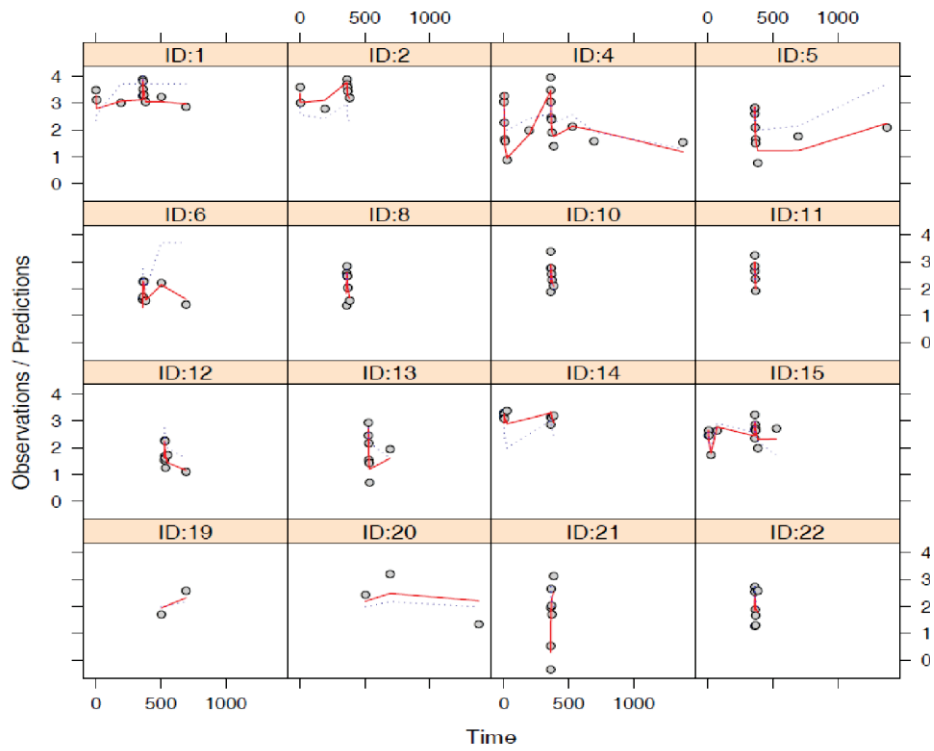
**Figure (11):** Diagnostic goodness-of-fit plot for the everolimus structural models, showing weighted residuals versus time (hours) after dose.



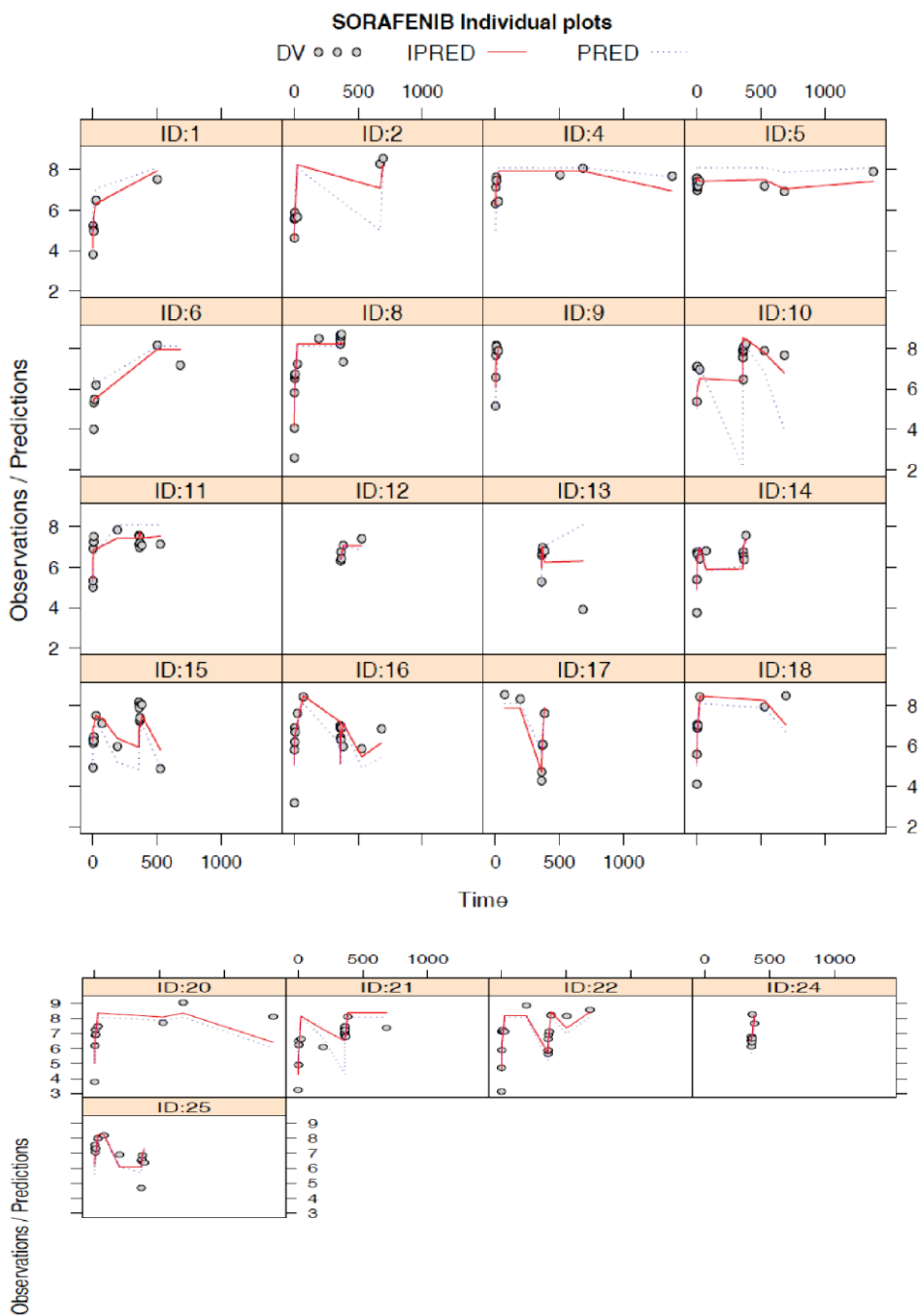
**Figure (12):** Visual predictive check for the structural model of sorafenib with the median, 75<sup>th</sup>, and 25<sup>th</sup> predicted and observed percentiles. The dashed line represents the observed median concentrations of drug doses. The solid line represents the predicted median concentrations of drug doses. The shaded areas around the prediction intervals represent the 95% confidence intervals (CI) around each of the prediction percentiles.



**Figure (13):** Visual predictive check for the structural model of everolimus, with the median, 75<sup>th</sup>, and 25<sup>th</sup> predicted and observed percentiles. The dashed line represents the observed median concentrations of drug doses. The solid line represents the predicted median concentrations of drug doses. The shaded areas around the prediction intervals represent the 95% confidence intervals around each of the prediction percentiles.



**Figure (14):** Individual plots: everolimus vs time (Hr). Small circles represent observed concentrations, red lines represent individual predicted, dotted lines represent population predicted.



**Figure (15):** Individual plots: sorafenib vs Time (Hr). Small circles represent observed concentrations, red lines represent individual predicted, dotted lines represent population predicted.

### Pharmacokinetic interactions between sorafenib and everolimus

Addition of everolimus to sorafenib resulted in a non-significant decrease of sorafenib exposure described by reduced AUC median values (29.15mg.h/L) of “sorafenib + everolimus” group as compared to “sorafenib” group (42.9 mg.h/L), while C<sub>max</sub> values remain almost unchanged at 0.3 ug/ml and 0.8 ug/ml for ” sorafenib + everolimus” group and “sorafenib” group respectively. Similarly, there was a non-significant decrease in the level of C<sub>max</sub> after addition of sorafenib to everolimus: “sorafenib + everolimus” group (9.88ug/ml) as compared to “everolimus” group (12.4 ug/ml). Our study confirms that changing the sequential addition of either drug sorafenib or everolimus to the other drug didn’t result in any significant change in the pharmacokinetic profiles regarding PK parameters such as CL, V<sub>d</sub>, AUC and C<sub>max</sub>. -.

**Table (16): PK interaction between different treatment groups association of sorafenib and everolimus**

Treatment Groups	Everolimus Median (min-max)	Everolimus + Sorafenib Median (min-max)	Wilcoxon test	Sorafenib Median (min-max)	Sorafenib + Everolimus Median (min-max)	Wilcoxon Test
Cl L/h	3.19 (0.98-7.83)	4.6 (0.82-16.40)	0.67	4.88 (1.64-11.1)	6.40 (4.31-8.49)	0.91
Vd L	113.62 (107-190)	165 (107-205)	0.18	255 (55.1-74.8)	237 (176-298)	0.56
AUC mg.h/L	1.8 (0.64- 5.13)	1.2 (0.28-6.04)	0.85	42.9 (18.1 -69.3)	29.15 (11.8-46.5)	0.83
Cmax µg/L	12.4 (0.21-9.30)	9.88 (2.18-45.93)	0.78	0.31 (0.03-3.22)	0.28 (0.03-0.30)	0.74

### Correlations between pharmacokinetic parameters and toxicity

The risk of adverse events was higher in the continuous arms (A and B) than in the intermittent arms (C and D) (64.1% vs.35.9%,  $P < 0.0001$ ). No correlations were found between the different administration schedules and adverse events of all grades, when considered in subclasses as described in Tables 17 - 21. The association between administration schedules and toxicity outcome was measured by the calculation of odd ratio (OR). Our

results suggest that exposure to different administration schedules is associated with lower odds of toxicities as interpreted by OR values to be 0 (less than 1) for all the subclasses of toxicities except for cutaneous toxicities where exposure to schedule B was associated with higher odds values of cutaneous toxicities (rash) calculated to be 5.6 (more than 1) which might be explained that the risk of occurrence of rash is higher in administration schedule B.

Moreover, no correlations were identified between the calculated pharmacokinetic parameters (AUC and C<sub>max</sub>) of each drug and related toxicities. These results suggest that changing the dosing regimen of the two combined drugs, according to administration schedules A, B, C, and D, had no impact on the overall toxicity outcomes.



**Table (17) : Comparison of biological toxicities\* between different treatment schedules**

Treatment Arm	Odd Ratio	p-value
B	0	0.997
C	0	0.998
D	0	0.999

Biological toxicities\* is a subclass of toxicity consisting of thrombopenia, hypophosphoremia, transaminase elevation, Proteinurea, Hyperbilirubinemia.

**Table (18): Comparison of clinical toxicities\* between different treatment schedules**

Treatment Arm	Odd Ratio	p-value
B	0.29	0.241
C	0	0.997
D	0	0.996

Clinical toxicities is a subclass of toxicity consisting of fatigue, hand-foot syndrome, anorexia, hypertension, anemia, abdominal pain, fever.

**Table (19): Comparison of gastric toxicities\* between different treatment schedules.**

Treatment Arm	Odd Ratio	p-value
B	0.67	0.71
C	0	0.997
D	0.33	0.34

Gastric toxicities is a subclass of toxicity consisting of diarrhea and constipation.

**Table (20): Comparison of cutaneous toxicities\* between different treatment schedules**

Treatment Arm	Odd Ratio	p-value
B	5.6	0.172
C	0	0.997
D	0	0.996

Cutaneous toxicities is a subclass of toxicity consisting of rash

**Table (21): Comparison of uncommon toxicities\* between different treatment schedules.**

Treatment Arm	Odd Ratio	p-value
B	0.24	0.29
C	0	0.996
D	0	0.997

Uncommon toxicities is a subclass of toxicity consisting of hemolysis and pneumopathy.

**Table (22): Correlation between toxicities subclasses and estimated PK parameters**

EI	AUC_sora Estimated Coefficient	Cmax_sora Estimated Coefficient	AUC_eve Estimated Coefficient	Cmax_eve Estimated Coefficient
Biological Toxicities	1.82e <sup>-14</sup> (p=1)	-6.73e <sup>-18</sup> (p=1)	4.03e <sup>-15</sup> (p=1)	-7.56 e <sup>-16</sup> (p=1)
Clinical Toxicities	1.29e <sup>-14</sup> (p=1)	-6.22e <sup>-18</sup> (p=1)	3.51e <sup>-15</sup> (p=1)	6.39e <sup>-15</sup> (p=1)
Gastric Toxicities	2.18 e <sup>-15</sup> (p=1)	-6.38 e <sup>-19</sup> (p=1)	-8.06 e <sup>-16</sup> (p=1)	-1.71e <sup>-15</sup> (p=1)
Cutaneous Toxicities	-0.054779 (p=0.973)	0.004812 (p=0.156)	-1.569877 (p=0.42)	0.354068 (p=0.834)
Uncommon Toxicities	6.39 e <sup>-19</sup> (p=1)	-1.63 e <sup>-14</sup> (p=1)	4.69e <sup>-15</sup> (p=1)	6.04 e <sup>-15</sup> (p=1)

AUC\_sora: Area Under The Curve of sorafenib

AUC\_eve: Area Under The Curve of everolimus

Cmax\_sora: Maximum Concentration of sorafenib

Cmax\_eve: Maximum Concentration of everolimus

Estimated Coefficient=  $\exp(\beta)$ . The coefficient  $\beta$  of the exposure variable in logistic regression model is the logarithm of odd-ratio measuring the association between that variable and the adverse events.

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## **Pharmacodynamics**

### **Serum biomarkers**

The effects of the association of everolimus (5mg qd po) and sorafenib (200 mg bid po) according to different administration schedules A, B, C & D on serum concentrations of different tumor biomarkers is described as follow:

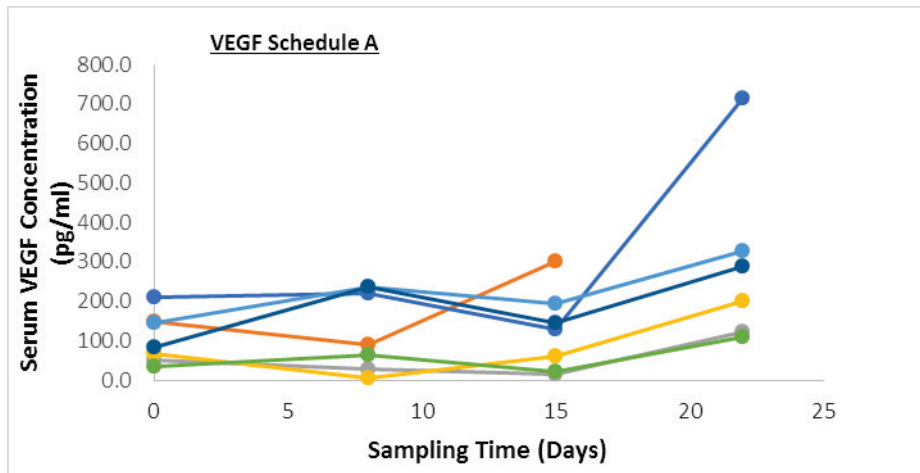
### **Serum Vascular Endothelial Growth Factor (VEGF)**

**In Schedule A:** serum VEGF concentration decreased after the first week of everolimus administration. Starting from day 15 after addition of sorafenib, VEGF concentrations were 1.5 to 4.0- fold higher than treatment with everolimus alone. Figure 16 (a).

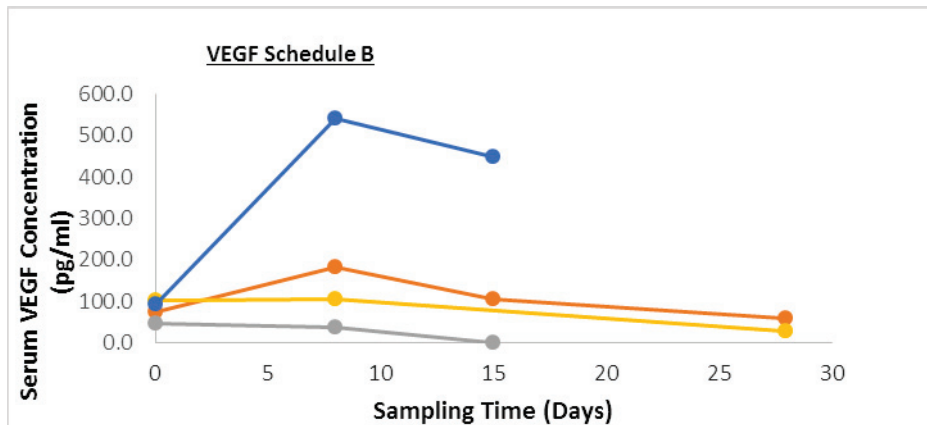
**In Schedule B:** serum VEGF concentration increased immediately after treatment with sorafenib and then decreased gradually starting from day 7 till day 28 of cycle 1. Concentration decreased by nearly 2 fold after addition of everolimus at day 15 as compared to treatment with sorafenib alone. Figure 16(b)

**In Schedule C:** serum VEGF concentration slightly decreased for most patients starting from day 8 and decreased continuously after alternating treatment with everolimus till day 28 (end of cycle 1). Figure 16(c)

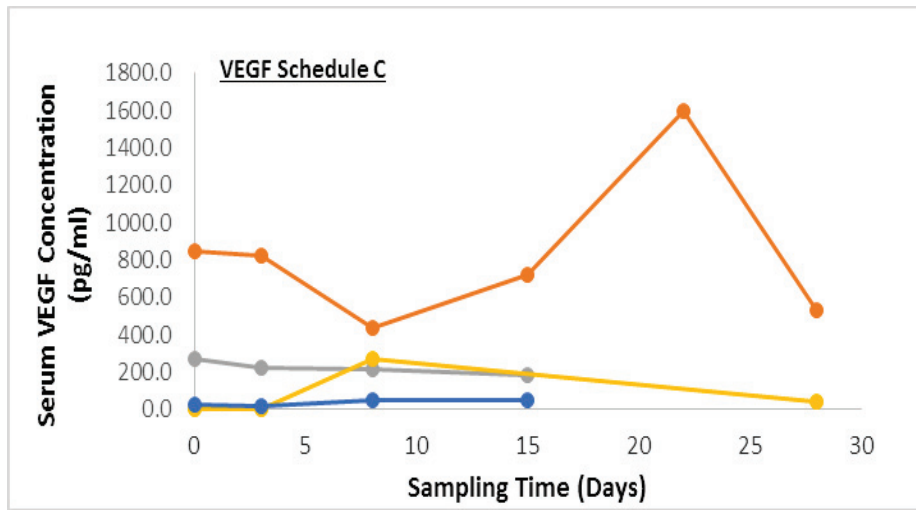
**In Schedule D:** serum VEGF concentrations slightly decreased after first week of treatment with everolimus and sorafenib 3 days on and 4 days off while increased at day 15 till the end of cycle 1 at day 28. Figure 16(d)



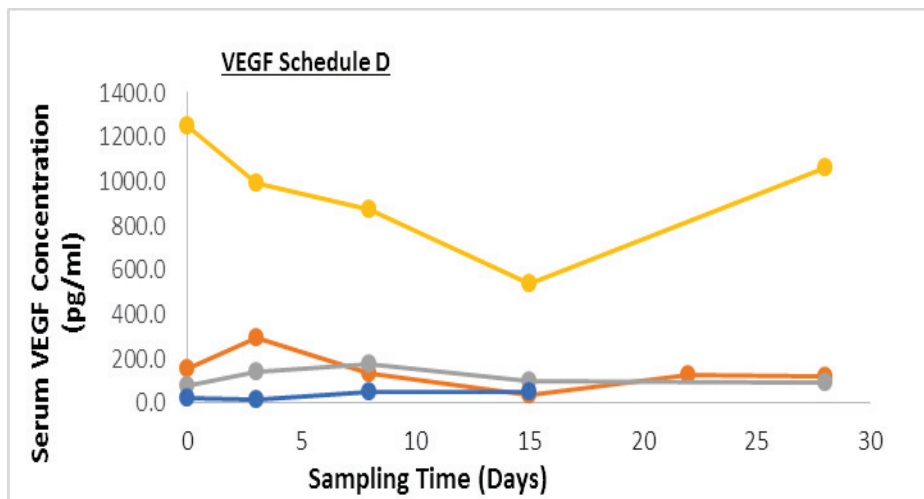
**Figure (16): (a):** VEGF biomarker profile of schedule A dosing regimen describing serum VEGF concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (16): (b):** VEGF biomarker profile of schedule B dosing regimen describing serum VEGF concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2.



**Figure (16): (c):** VEGF biomarker profile of schedule C dosing regimen describing serum VEGF concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (16): (d):** VEGF biomarker profile of schedule D dosing regimen describing serum VEGF concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2

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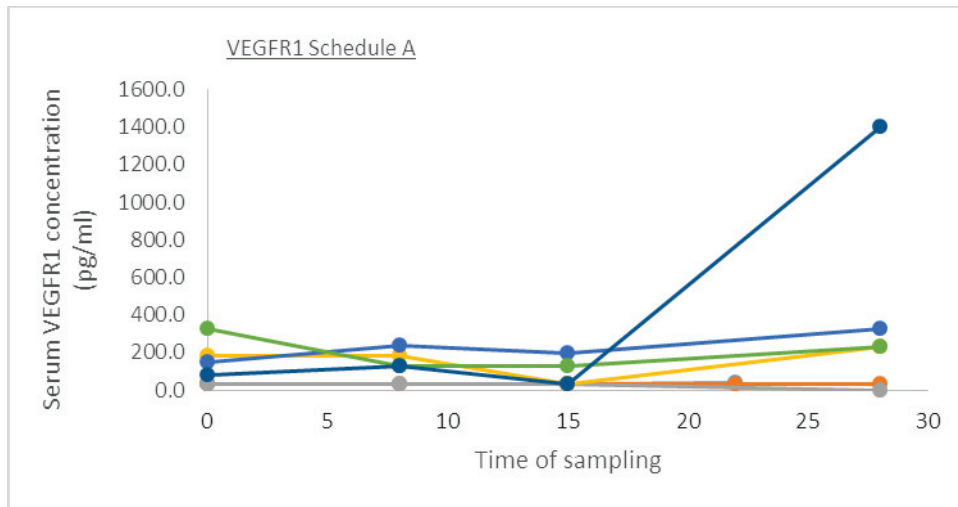
**Vascular Endothelial Growth Factor Receptor/ (fms-like tyrosine kinase-1 (VEGFR-1)**

**In Schedule A:** Acute post-treatment elevation of VEGFR1 concentration was observed after treatment with everolimus then decreased after the first week of everolimus administration. Starting from day 15 after addition of sorafenib, VEGFR-1 concentrations were 2.0 fold higher than treatment with everolimus alone. Figure 17(a).

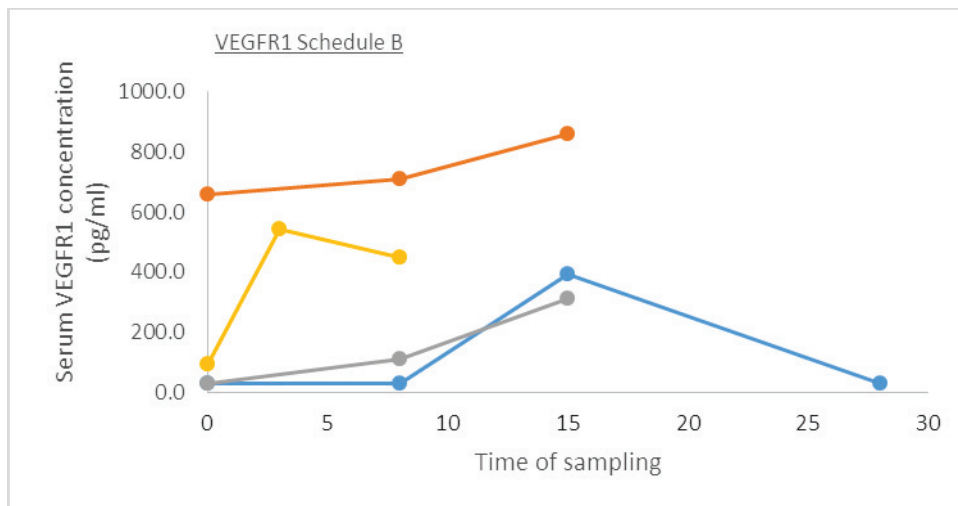
**In Schedule B:** VEGFR1 concentration increased immediately after treatment with sorafenib and then decreased gradually starting from day 15 till day 28 of cycle 1 treatment after addition of everolimus. Figure 17 (b).

**In Schedule C:** acute post-treatment decrease of VEGFR1 concentration after treatment with sorafenib on day 3 then it slightly increased starting from day 7 to day 15 for most patients after alternating therapy with everolimus. Figure 17 (c).

**In Schedule D:** acute post-treatment change of VEGFR1 concentrations after treatment of everolimus and sorafenib and then concentration slightly decreased after day 7 then remained unchanged till the end of cycle 1 at day 28. Figure 17 (d)

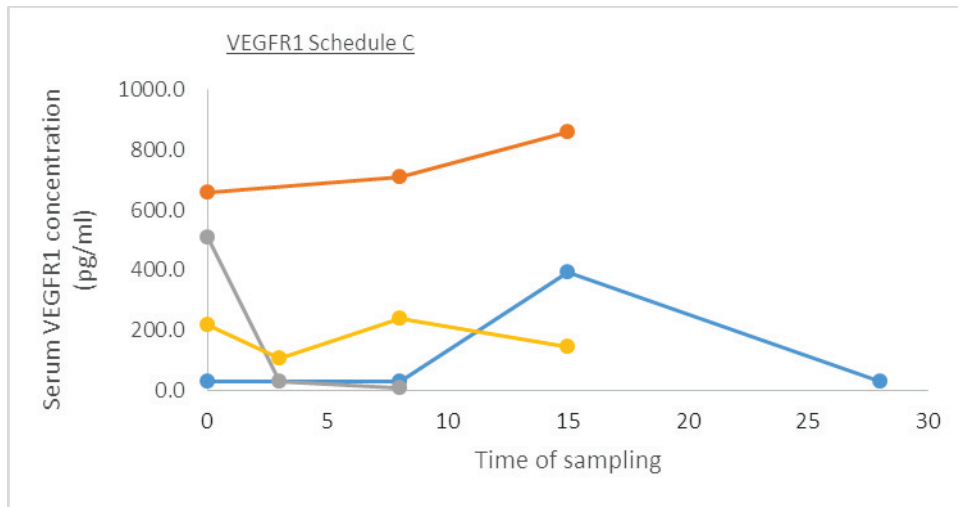


**Figure (17): (a):** VEGFR1 biomarker profile of schedule A dosing regimen describing serum VEGFR1 concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2

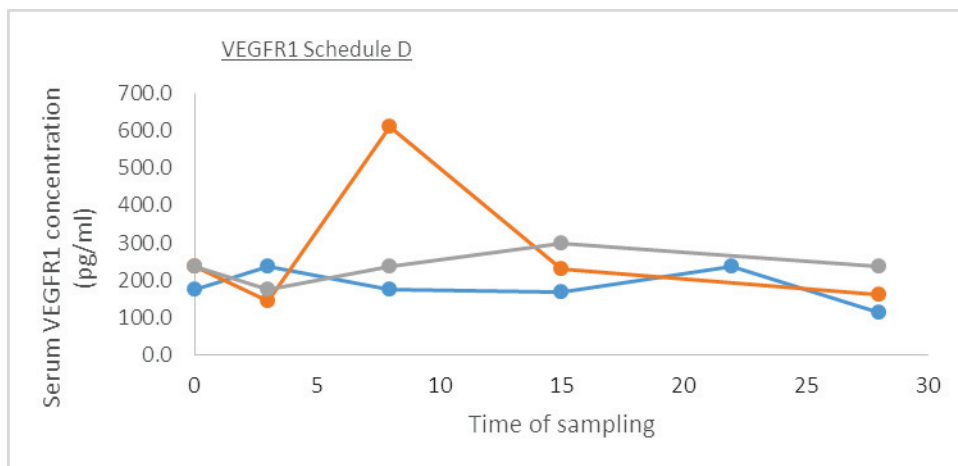


**Figure (17): (b):** VEGFR1 biomarker profile of schedule B dosing regimen describing serum VEGFR1 concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2





**Figure (17):** (c): VEGFR1 biomarker profile of schedule C dosing regimen describing serum VEGFR1 concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (17):** (d): VEGFR1 biomarker profile of schedule D dosing regimen describing serum VEGFR1 concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2

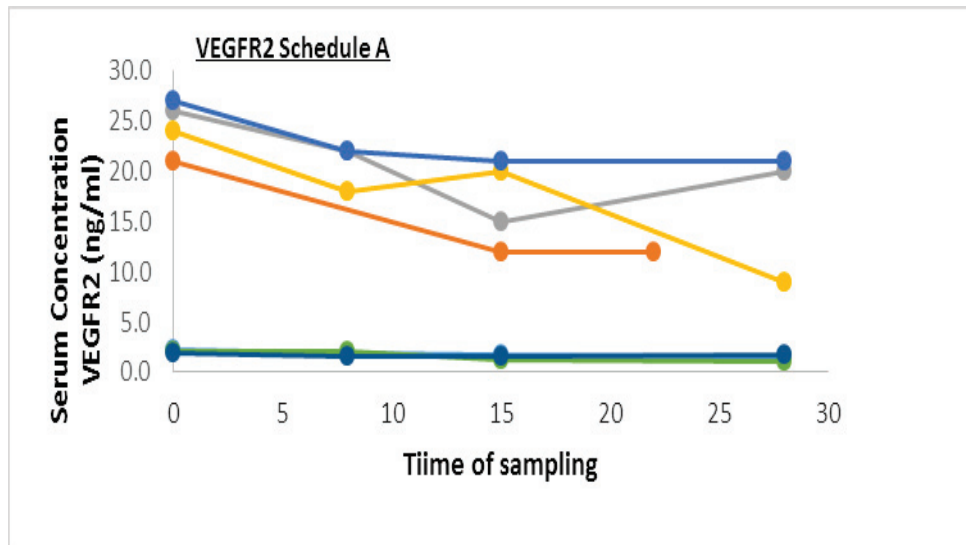
**Vascular Endothelial Growth Factor Receptor/(Flk-1, fetal liver kinase) (VEGFR -2)**

**In Schedule A:** VEGFR-2 concentration decreased gradually after the first week of treatment with everolimus. Starting from day 15 after addition of sorafenib, VEGFR-2 concentrations slightly increased or remained unchanged as compared to everolimus alone. Figure 18 (a).

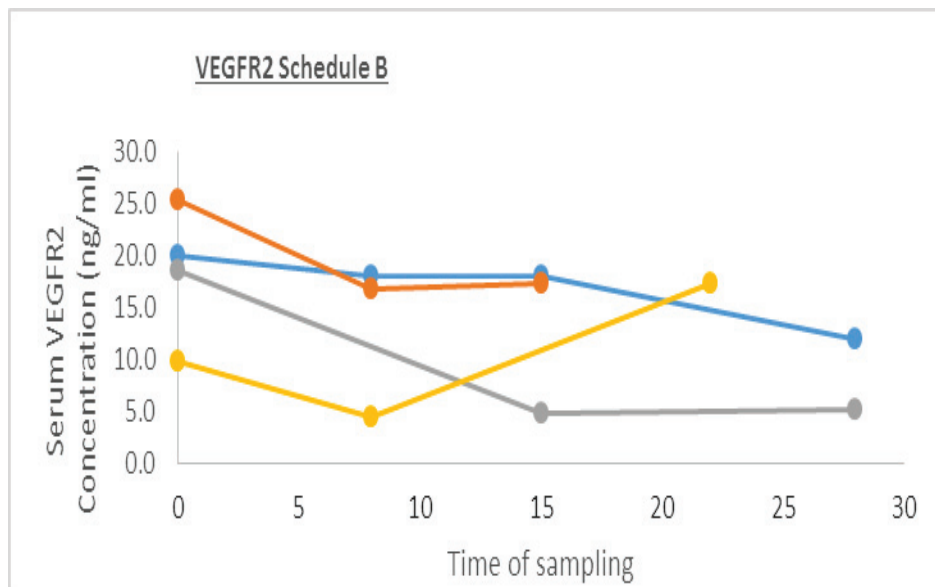
**In Schedule B:** VEGFR-2 concentration decreased gradually after treatment with sorafenib then slightly decreased starting from day 15 till day 28 of cycle 1 treatment after addition of everolimus. Figure 18 (b).

**In Schedule C:** acute post-treatment decrease of VEGFR-2 concentration after treatment with sorafenib on day 3 then a slightly increased starting from day 7 to day 15 for most patients after alternating therapy with everolimus. Figure 18 (c)

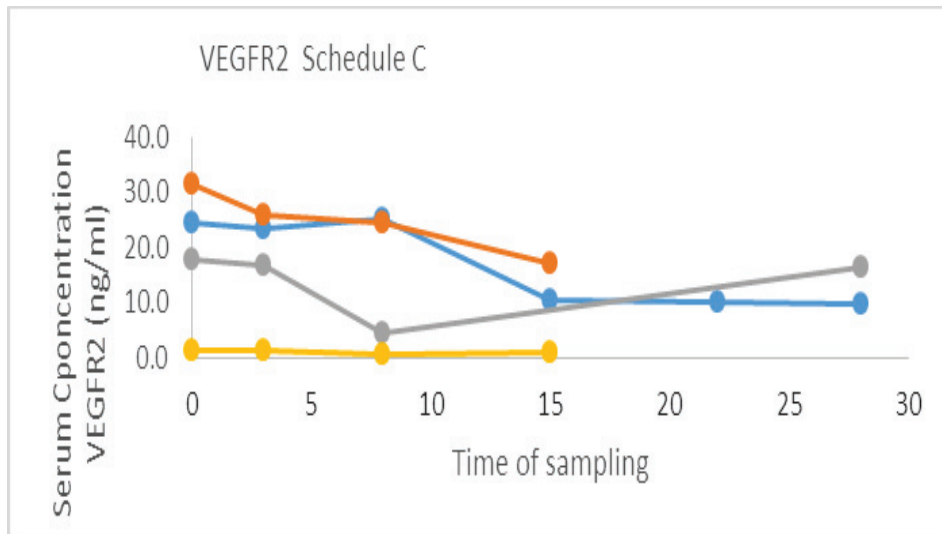
**In Schedule D:** acute post-treatment increase of VEGFR-2 after treatment with everolimus and sorafenib and then VEGFR concentrations slightly decreased after day 7 then remain unchanged till the end of cycle 1 at day 28. Figure 18 (d)



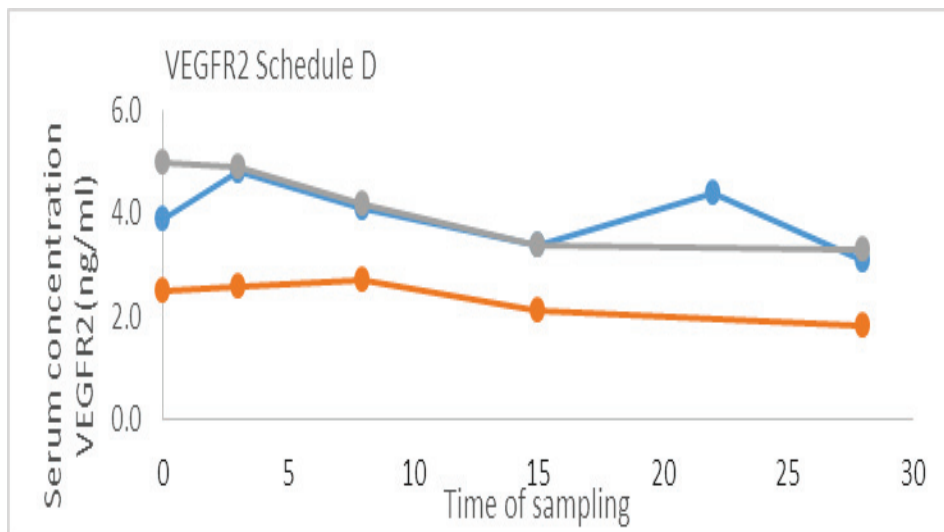
**Figure (18):** (a).: VEGFR2 biomarker profile of schedule A dosing regimen describing serum VEGFR1 concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (18):** (b).: VEGFR2 biomarker profile of schedule B dosing regimen describing serum VEGFR2 concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (18):** (c): VEGFR2 biomarker profile of schedule C dosing regimen describing serum VEGFR2 concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2.



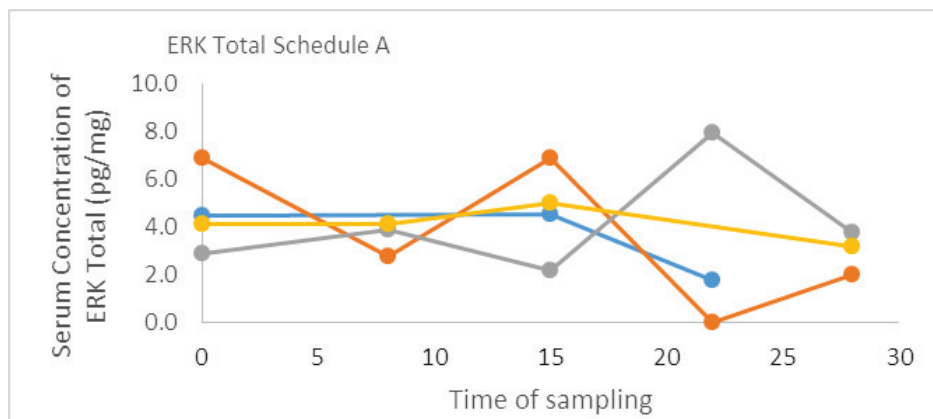
**Figure (18):** (d): VEGFR2 biomarker profile of schedule D dosing regimen describing serum VEGFR2 concentration (pg/ml) measured at different time points of sampling taken during cycle 1 and cycle 2.

### **Total Extracellular signal-regulated kinase (ERK)**

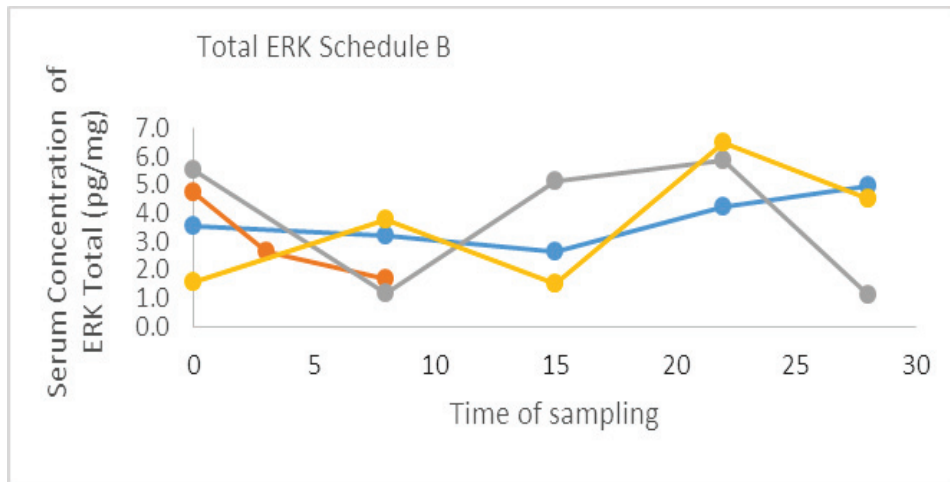
**In Schedule A** ERK total concentration gradually increased during the first week of treatment with everolimus. Starting from day 15 after addition of sorafenib, ERK total concentration decreased by more than 2 fold as compared to everolimus alone. Figure 19 (a).

**In Schedule B:** ERK total concentration decreased gradually during the first week of treatment with sorafenib then continue to decrease till day 15. ERK total concentration then increased after addition of everolimus during one week then decreased again at day 28 of cycle 1 Figure 19 (b).

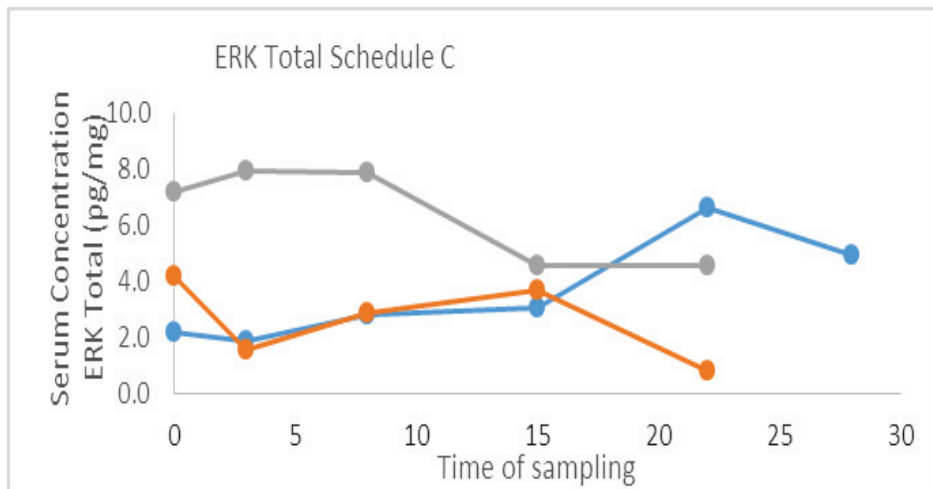
**In Schedule C** acute post-treatment decrease of ERK total concentration after treatment with sorafenib on day 3 then gradually increased during the first week of treatment till day 15 after alternating therapy with everolimus. ERK total concentration increased or remain unchanged for the rest of the treatment cycle when alternating sorafenib then after on day 28. Figure 19 (c)



**Figure (19):** (a): ERK Total biomarker profile of schedule A dosing regimen describing serum ERK Total concentration (pg/mg) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (19): (b):** ERK Total biomarker profile of schedule B dosing regimen describing serum ERK Total concentration (pg/mg) measured at different time points of sampling taken during cycle 1 and cycle 2.



**Figure (19): (c):** ERK Total biomarker profile of schedule C dosing regimen describing serum ERK Total concentration (pg/mg) measured at different time points of sampling taken during cycle 1 and cycle 2.

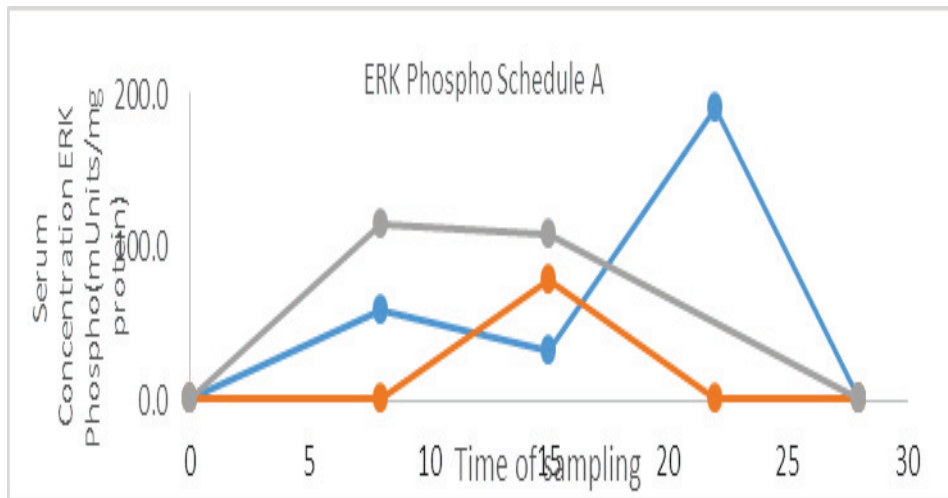
**Phosphorylated Extracellular signal-regulated kinase (p-ERK)**

**In Schedule A:** p-ERK concentration decreased gradually after the first week of treatment with everolimus then sharply decreased starting from day 15 after addition of sorafenib. Figure 20 (a).

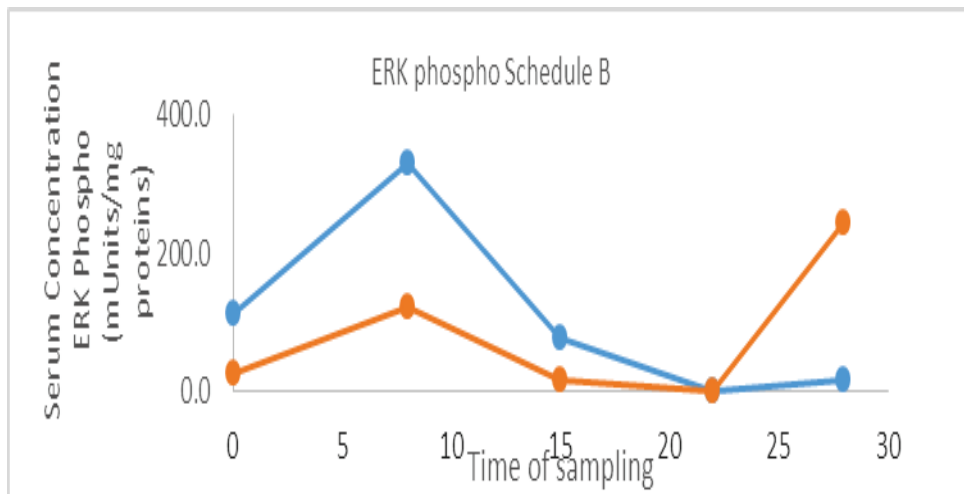
**In Schedule B:** p-ERK concentration increased after treatment with sorafenib then gradually decreased starting from day 15 till day 28 of cycle 1 treatment after addition of everolimus. Figure 20 (b).

**In Schedule C:** p-ERK decreased concentration during the first week of treatment with sorafenib for most patients while concentrations increased after alternating therapy with everolimus on day 7 and increased continuously after addition of sorafenib on day 15. Figure 20 (c)

**In Schedule D:** p-ERK concentration increased after day 15 then remain unchanged till the end of cycle 1 at day 28. Figure 20 (d)

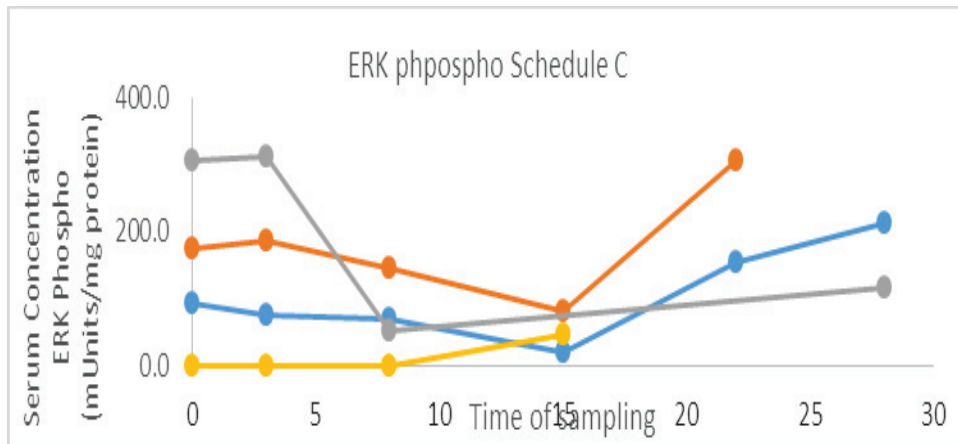


**Figure (20):** (a.): ERK phosphorylated biomarker profile of schedule A dosing regimen describing serum ERK phosphorylated concentration (mUnits/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2

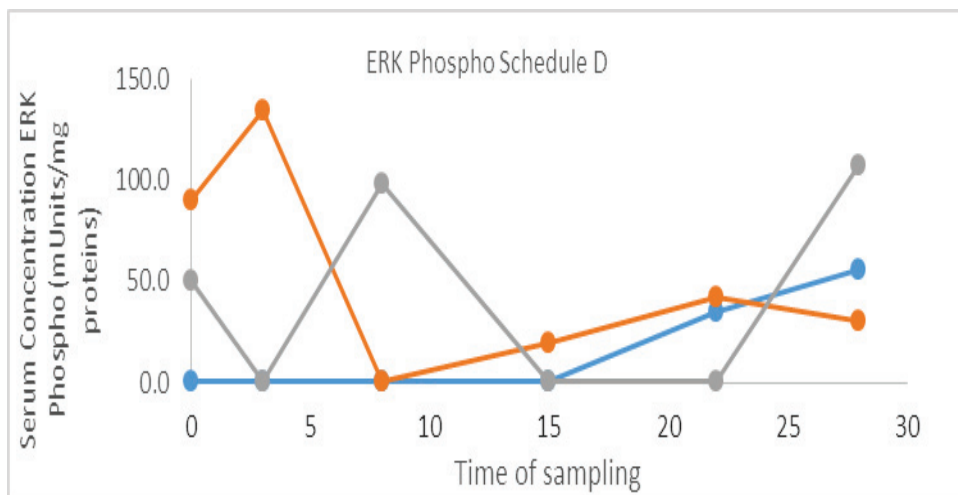


**Figure (20):** (b.): ERK phosphorylated biomarker profile of schedule B dosing regimen describing serum ERK phosphorylated concentration (mUnits/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2





**Figure (20):** (c): ERK phosphorylated biomarker profile of schedule C dosing regimen describing serum ERK phosphorylated concentration (mUnits/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (20):** (d): ERK phosphorylated biomarker profile of schedule D dosing regimen describing serum ERK phosphorylated concentration (mUnits/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2

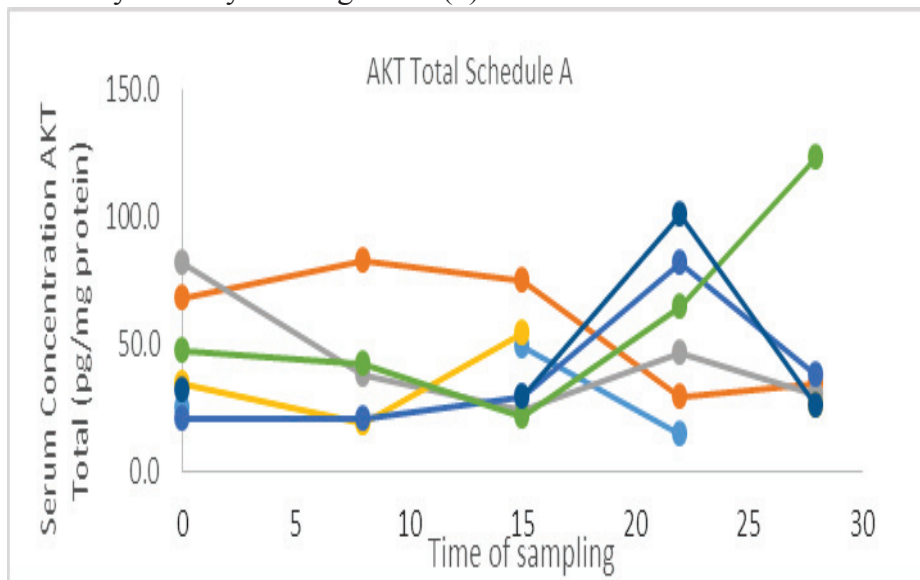
**Total Serine/threonine kinase (AKT)**

**In Schedule A:** AKT concentration increased starting from day 15 after addition of sorafenib and then decreased starting from day 22 till day 28 of cycle 1 Figure 21 (a).

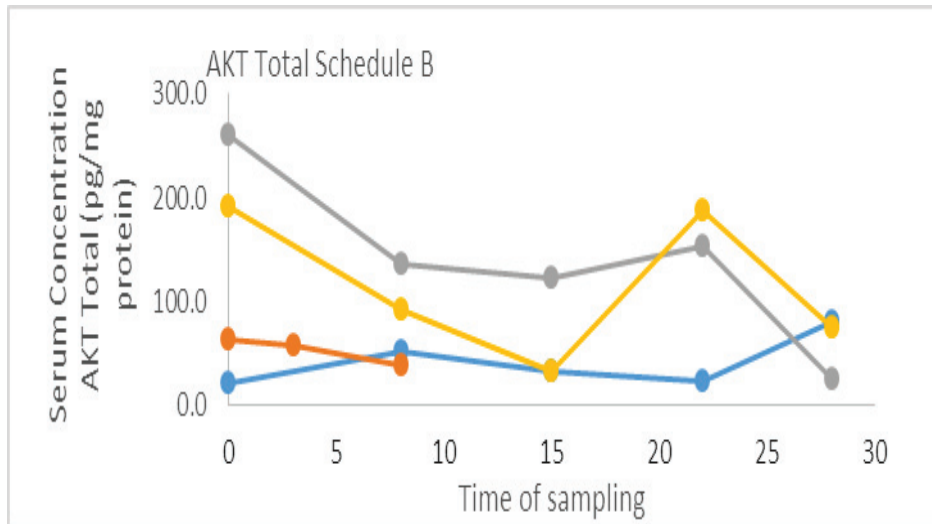
**In Schedule B:** AKT concentration decreased during first week treatment with sorafenib then gradually increased starting from day 15 after addition of everolimus. Figure 21 (b).

**In Schedule C:** There is a slight decrease of AKT concentration during the first week of treatment with sorafenib for most patients while concentrations remain unchanged after alternating therapy with everolimus on day 7 then decreased continuously after addition of sorafenib on day 15. Figure 21 (c)

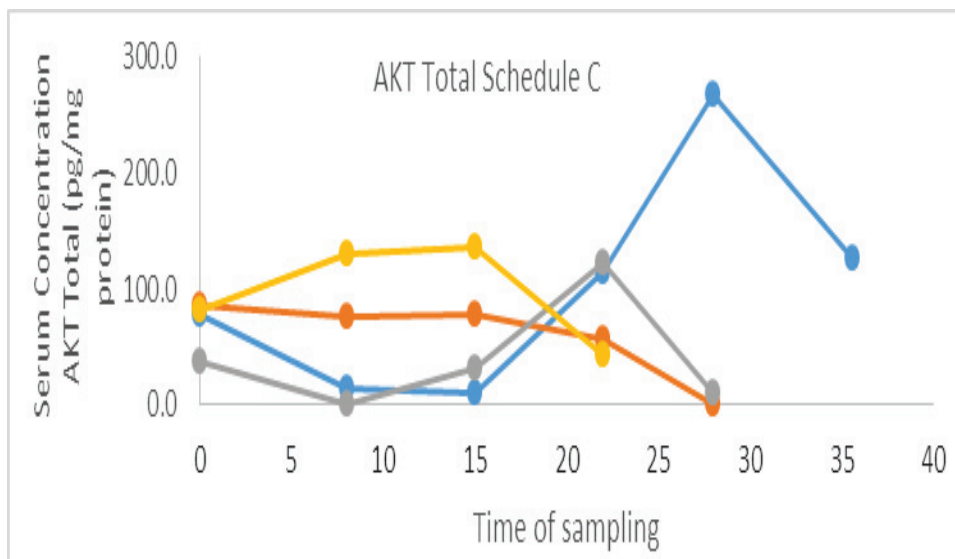
**In Schedule D:** AKT concentration slightly increased during first week treatment with everolimus and sorfenib, then decreased starting from day 15 till day 28 of cycle 1 Figure 21 (d)



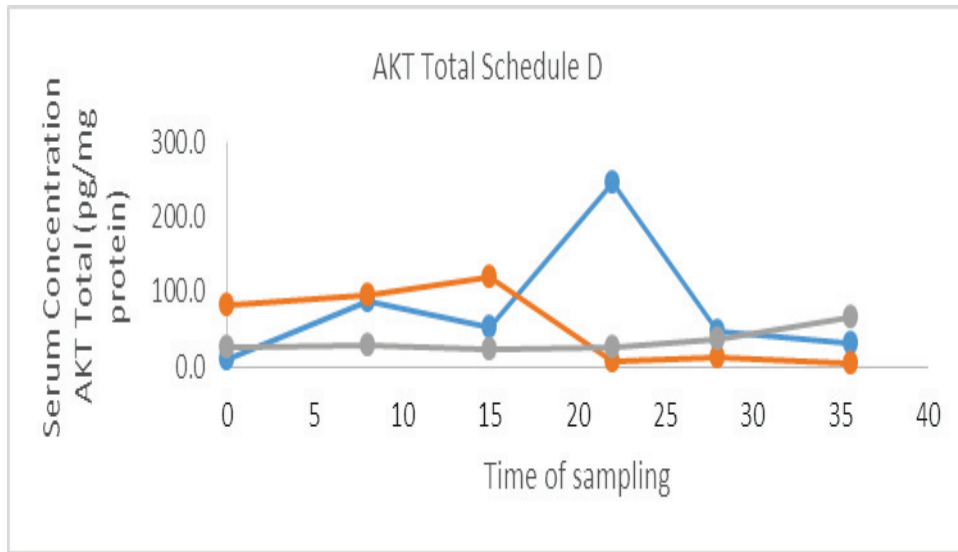
**Figure (21):** (a).: AKT Total biomarker profile of schedule A dosing regimen describing serum AKT Total concentration (pg/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (21):** (b).: AKT Total biomarker profile of schedule B dosing regimen describing serum AKT Total concentration (pg/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (21):** (c).: AKT Total biomarker profile of schedule C dosing regimen describing serum AKT Total concentration (pg/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2.



**Figure (21):** (d).: AKT Total biomarker profile of schedule D dosing regimen describing serum AKT Total concentration (pg/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2

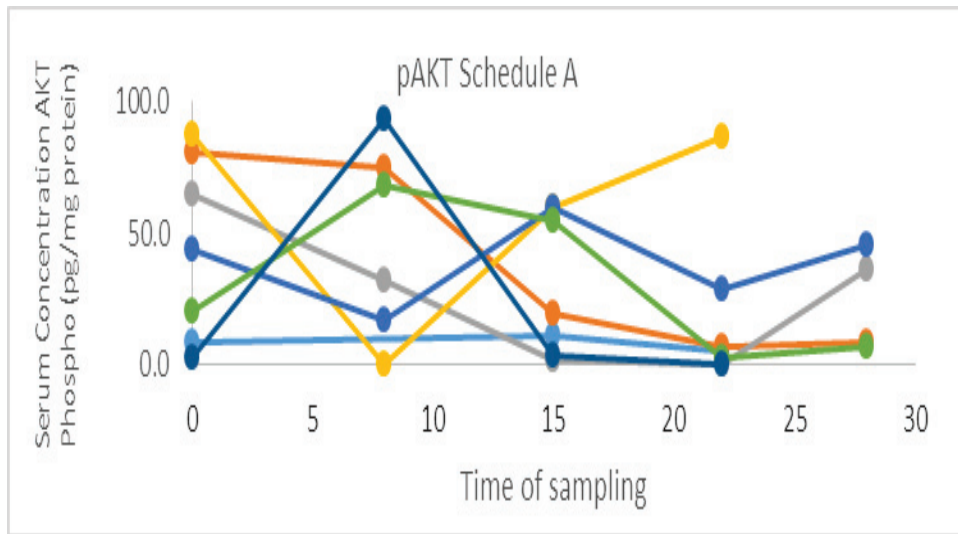
### **Phosphorylated Serine/threonine kinase (pAKT)**

**In Schedule A:** pAKT concentration decreased starting from day 15 after addition of sorafenib and then increased starting from day 22 till day 28 of cycle 1 Figure 22 (a).

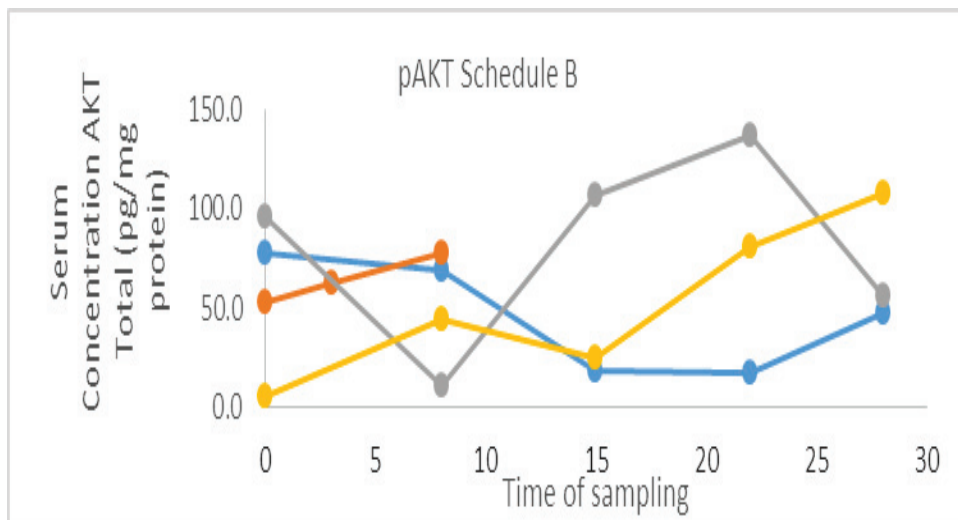
**In Schedule B:** pAKT concentration decreased on day 7 after treatment with sorafenib then gradually increased starting from day 15 after addition of everolimus till day 28 of cycle 1 Figure 22 (b).

**In Schedule C:** a slight decrease of pAKT concentration during the first week of treatment with sorafenib for most patients while concentrations remain unchanged after alternating therapy with everolimus on day 7 then increased continuously after addition of sorafenib on day 15. Figure 22 (c)

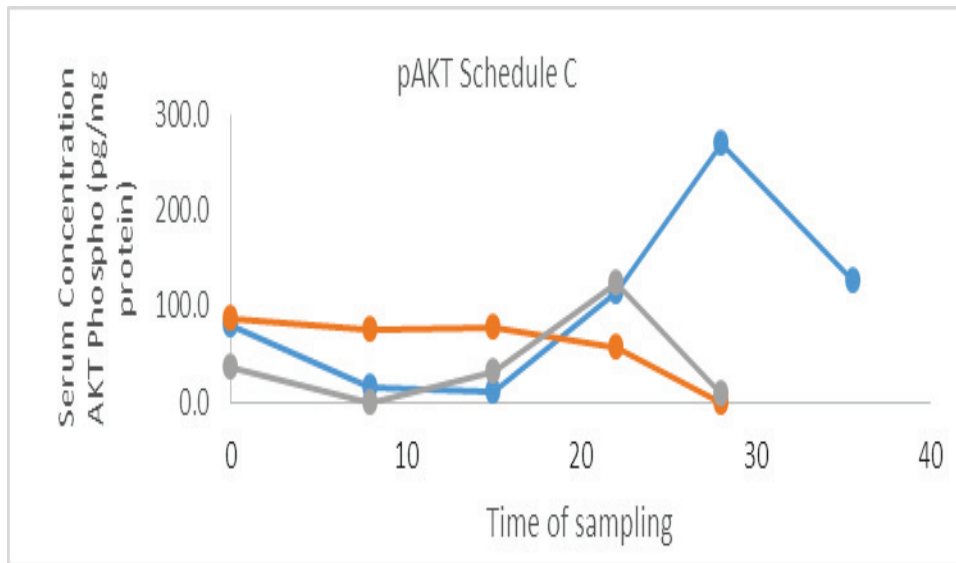
**In Schedule D:** pAKT concentration remain unchanged during the first week treatment with everolimus and sorfenib and then increased starting from day 22 till day 28 of cycle 1. Figure 22 (d)



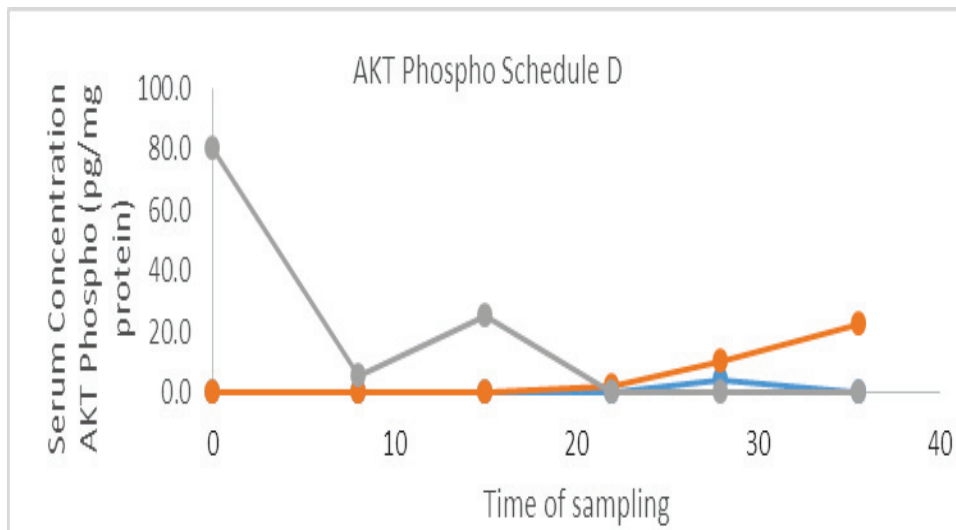
**Figure (22):** (a): pAKT biomarker profile of schedule A dosing regimen describing serum p AKT concentration (pg/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (22):** (b): pAKT biomarker profile of schedule B dosing regimen describing serum p AKT concentration (pg/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (22):** (c): pAKT biomarker profile of schedule C dosing regimen describing serum p AKT concentration (pg/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2.



**Figure (22):** (d): pAKT biomarker profile of schedule D dosing regimen describing serum p AKT concentration (pg/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2.

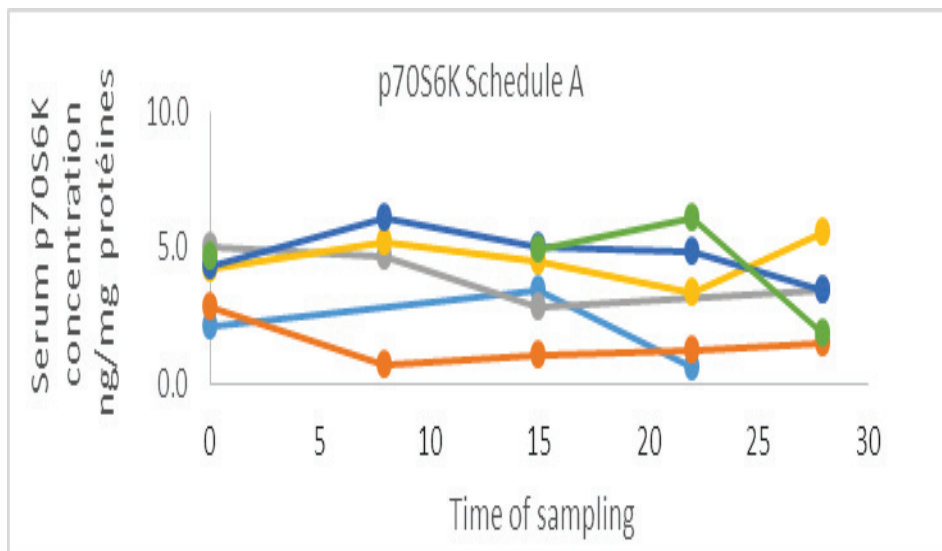
**70-kDa S6 protein kinase (p70S6K)**

**In Schedule A:** p70S6K concentration increased during first week of treatment then gradually decreased. Starting from day 15 after addition of sorafenib, p70S6K concentration slightly increased till day 28 of cycle 1. Figure 23 (a).

**In Schedule B:** p70S6K concentration decreased on day 7 after treatment with sorafenib then gradually increased starting from day 15 after addition of everolimus till day 28 of cycle 1 Figure 23 (b).

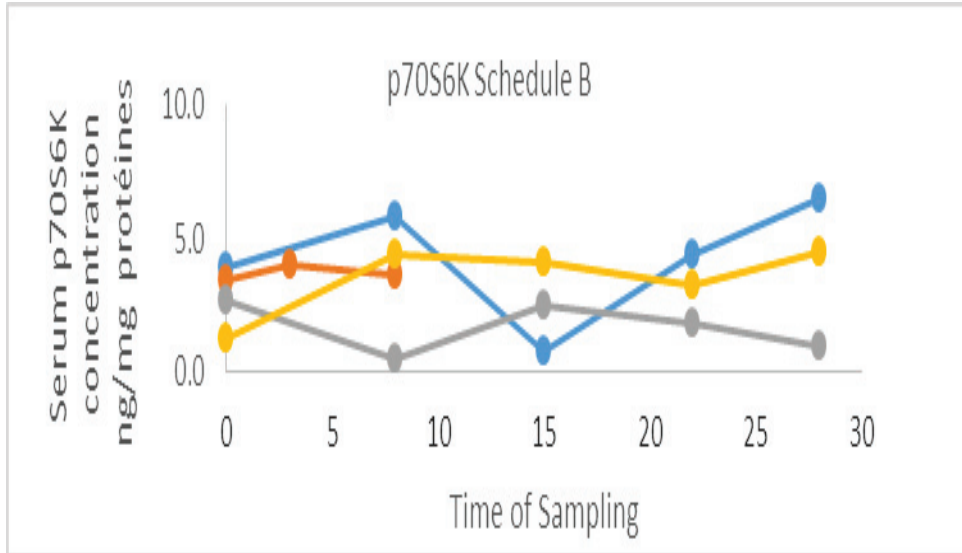
**In Schedule C:** There is a slight increase of p70S6K concentration on day 7 after alternating therapy with everolimus for most patients while concentrations decreased on day 15 upon addition of sorafenib then increased continuously on day 28 of cycle 1. Figure 23 (c)

**In Schedule D:** p70S6K concentration decreased during the first week of treatment with everolimus and sorfenib and then increased Starting from day 22 till day 28 of cycle 1. Figure 23 (d)

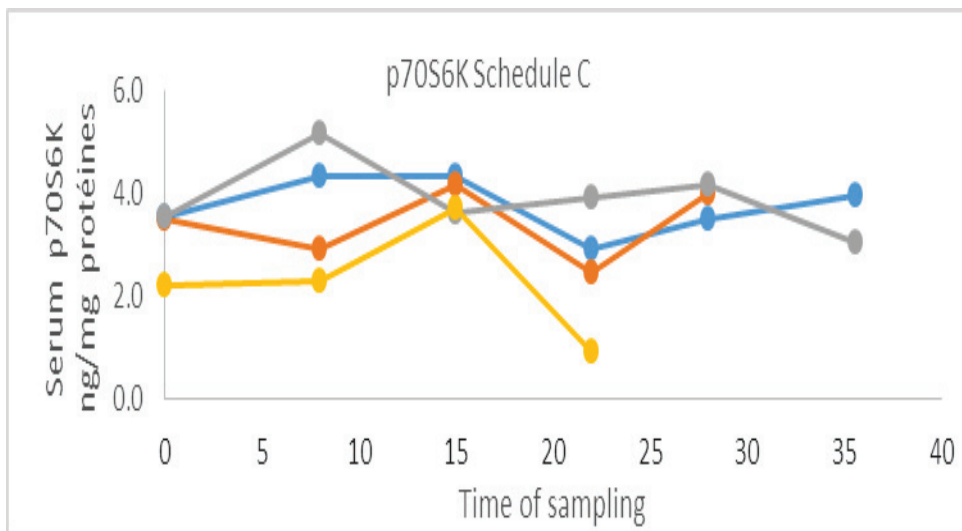




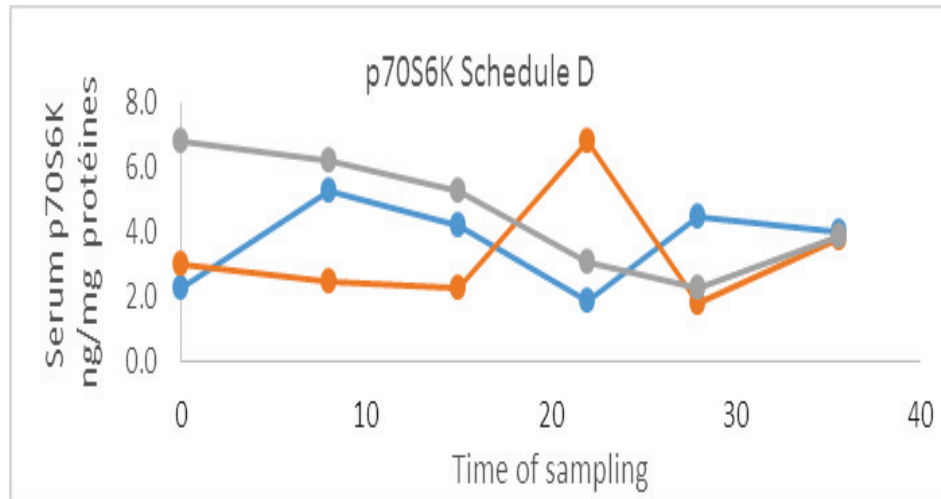
**Figure (23):** (a): p70S6K biomarker profile of schedule A dosing regimen describing serum p70S6K concentration (ng/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (23):** (b): p70S6K biomarker profile of schedule B dosing regimen describing serum p70S6K concentration (ng/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (23):** (c): p70S6K biomarker profile of schedule C dosing regimen describing serum p70S6K concentration (ng/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2



**Figure (23):** (d): p70S6K biomarker profile of schedule D dosing regimen describing serum p70S6K concentration (ng/mg protein) measured at different time points of sampling taken during cycle 1 and cycle 2

### **Evaluation of clinical activity**

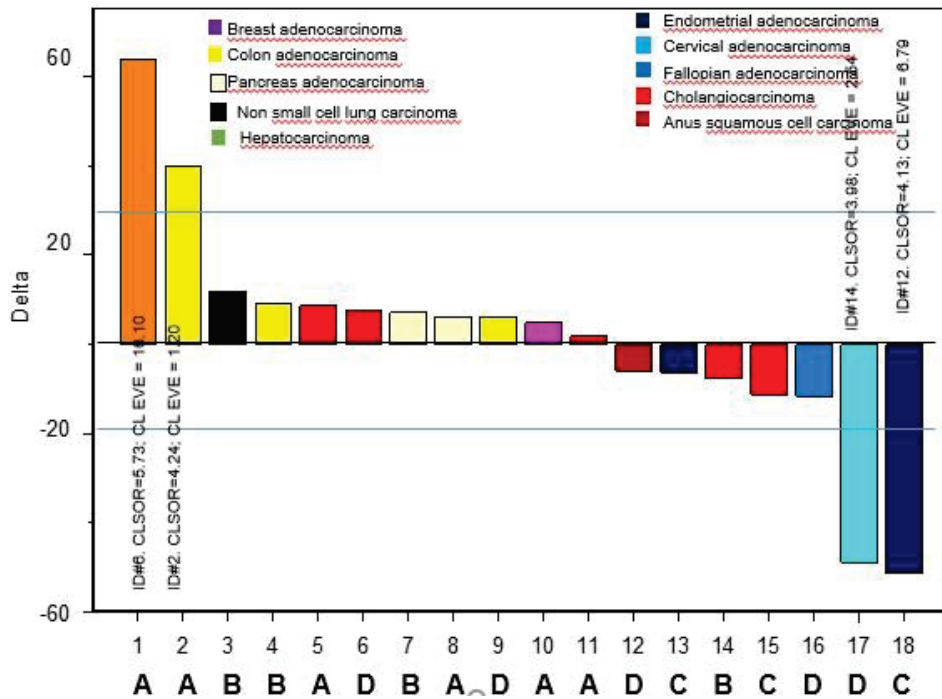
Total 18 out of 26 patients were assessable for tumor response. The remaining 8 patients were not assessable due to early study treatment discontinuation related to toxicity (n=4); infection (n=2) and early progression (n=2).

The best tumor responses were: partial response, 2/18 (11%); stable disease, 14/18 (78%); progressive disease, 2/11 (22%). The best tumor response rates were as follows in continuous dosing arms: progressive disease 2/10 (20%) and stable disease 8/10 (80%) (Arm A, progressive disease 2/6, stable disease 4/6; Arm B, stable disease 4/4). The best tumor responses rates were as follows in intermittent dosing arms: partial response

2/8 (25%) and stable disease 6/8 (75%): Arm C, partial response 1/3, stable disease 2/3; Arm D, partial response 1/5, stable disease 4/5.

Six patients experienced long stable diseases for more than 4 months (37.5%). Of note, among 8 patients with cholangiocarcinomas, 5 were assessable for tumor responses. All of them (100%) had stable disease, including 3 with long stability (20 months; 12 months and 5 months). Moreover, among 4 patients with gynecological adenocarcinomas, 2 experienced partial responses and 2 had stable diseases.

Measurement of tumor size performed by Imaging assessments of tumor response using RECIST v1.0 revealed a percent reduction of 6-52% of tumor size from baseline that were seen in 7 patients (38.8%) belonging to different administration schedules (Arm B=1/4; Arm C=2/3; Arm D 2/5) The most common pathological occurrence among these patients were cholangiocarcinomas, anus squamous cell carcinoma, cervical adenocarcinoma and endometrial adenocarcinoma.



**Figure (24):** Waterfall plot of best overall change from baseline in target lesion measurement by RECIST (Response Evaluation Criteria in Solid Tumors) guidelines for patients at different administration Schedule

**Correlation between pharmacokinetic parameters and clinical response**

The clinical response categorized either as stable disease (SD) > 6 months, or non responders (PD), was examined in relation to drug exposure, in terms of Cmax and AUC of both drugs, sorafenib and everolimus. Regression analysis suggested that there is a high risk of association between drug exposure and clinical response, the odd ratio corresponding to AUC sorafenib, and AUC everolimus is two times greater in responders (SD) compared to non responders.

Similarly, the odd ratio corresponding to Cmax sorafenib is estimated to be about four times greater in responders(SD) compared to non responders.

On the other hand, the odd ratio of Cmax everolimus was 0.1 (<1) which means that exposure to everolimus is associated with lower risk of response

**Table (23):** Correlation between PK parameters and clinical response

PK parameter	OR clinical response	Lower limit	Upper limit	p value
AUC_sorafenib	2.08	0.39	12.84	0.41
Cmax_sorafenib	4.71	0.82	39	0.10
AUC_everolimus	2.14	0.39	13.61	0.39
Cmax_everolimus	0.1	0	0.78	0.05441

### Correlation between biomarker profile and clinical activity

Our results show statistical significance between the two median values of PD and SD corresponding to mean serum concentration versus time of VEGF and PS6K biomarkers as p value of VEGF and PS6k were 0.0001846\*\*\*and 0.01498\*\* respectively as described in Figure 25. This indicates that VEGF and PS6K are the most sensitive biomarkers to treatment with sorafenib and everolimus combination in patients experienced a clinical response of stable disease. In contrary, the slope value calculated for each analyzed biomarker didn't show any significant change from responsive to progressive patients This was statistically assessed using Wilcoxon test that showed no statistical significance ( $p < 0.05$ ) between the two median slope values of PD and SD as described in table 24 and Figure 26. We found that VEGF values increased for stabilized patients as for progressive patients translated by positive values of VEGF slopes While, PS6K values decreased stabilized patients as for progressive patients translated by negative values of VEGF slopes This finding suggest that

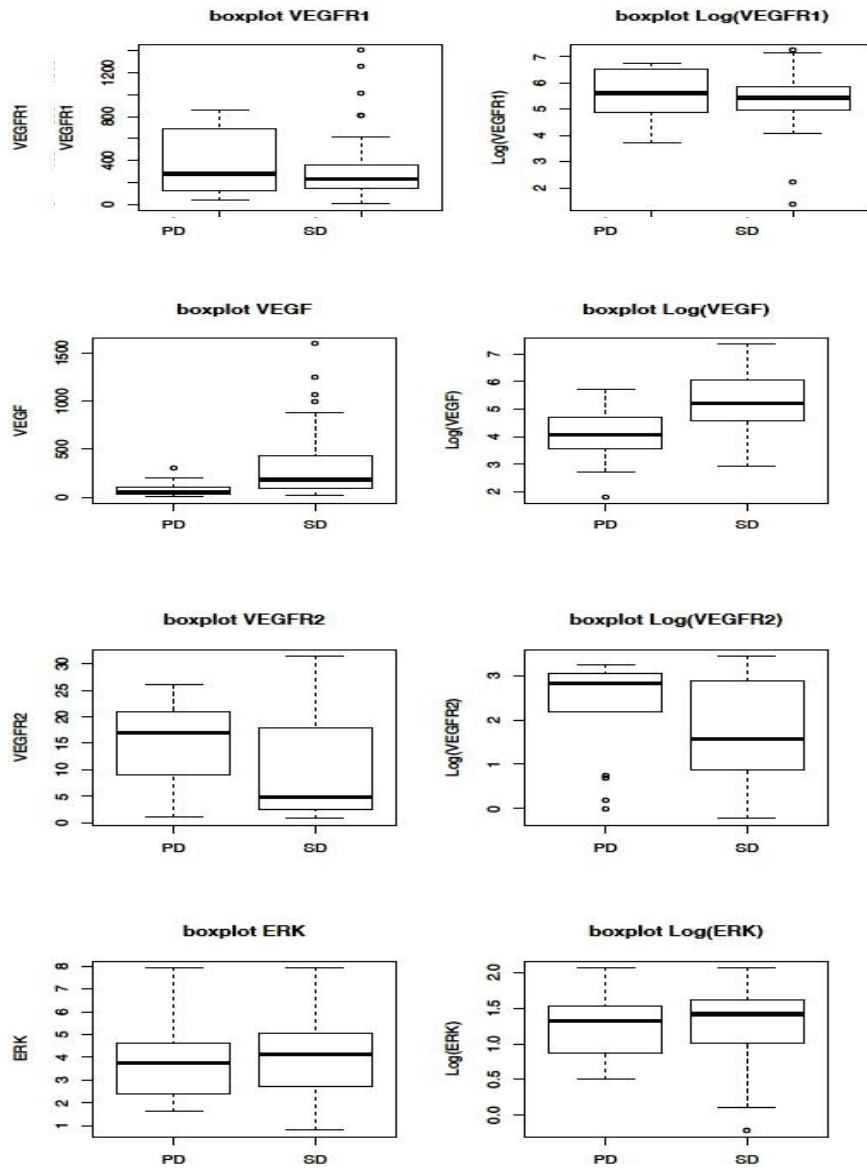
using mean serum concentration values of biomarkers to study correlation might be a more precise tool to identify the relationship between biomarker and response. On the other hand, slopes could be a useful tool to describe the kinetics of biomarkers as demonstrated in our results.

**Table (24): Correlation between biomarker concentration and clinical response.**

Tumor biomarker	Progressive disease (Median values)	Stable disease (Median values)	Wilcoxon (p-value)
VEGF	104.4	146.2	0.0001846***
VEGFR1	79	230.1	0.7327
VEGFR2	9.4	4.8	0.1014
ERK	4	4.1	0.7961
AKT	38	54.3	0.6294
PAKT	1.1	0.9	0.2298
PS6K	3.4	3.9	0.01498**

We studied the correlation between the median slope values corresponding to either PD or SD clinical response of serum concentrations of each of the following biomarker VEGF, VEGFR1, VEGFR2, AKT, pAKT, total S6K, ERK total, pERK using Boxplot representing different concentration versus time values and log concentration versus time values of all biomarkers were plotted against clinical response (SD or PD) followed by statistical analysis using Wilcoxon test that confirmed a statistical significance ( $p < 0.05$ ) between the two median values of PD and SD corresponding to concentration versus time of VEGF and PS6K

biomarkers as p value of VEGF =0.0001846\*\*\*and p value of PS6k 0.01498\*\*.



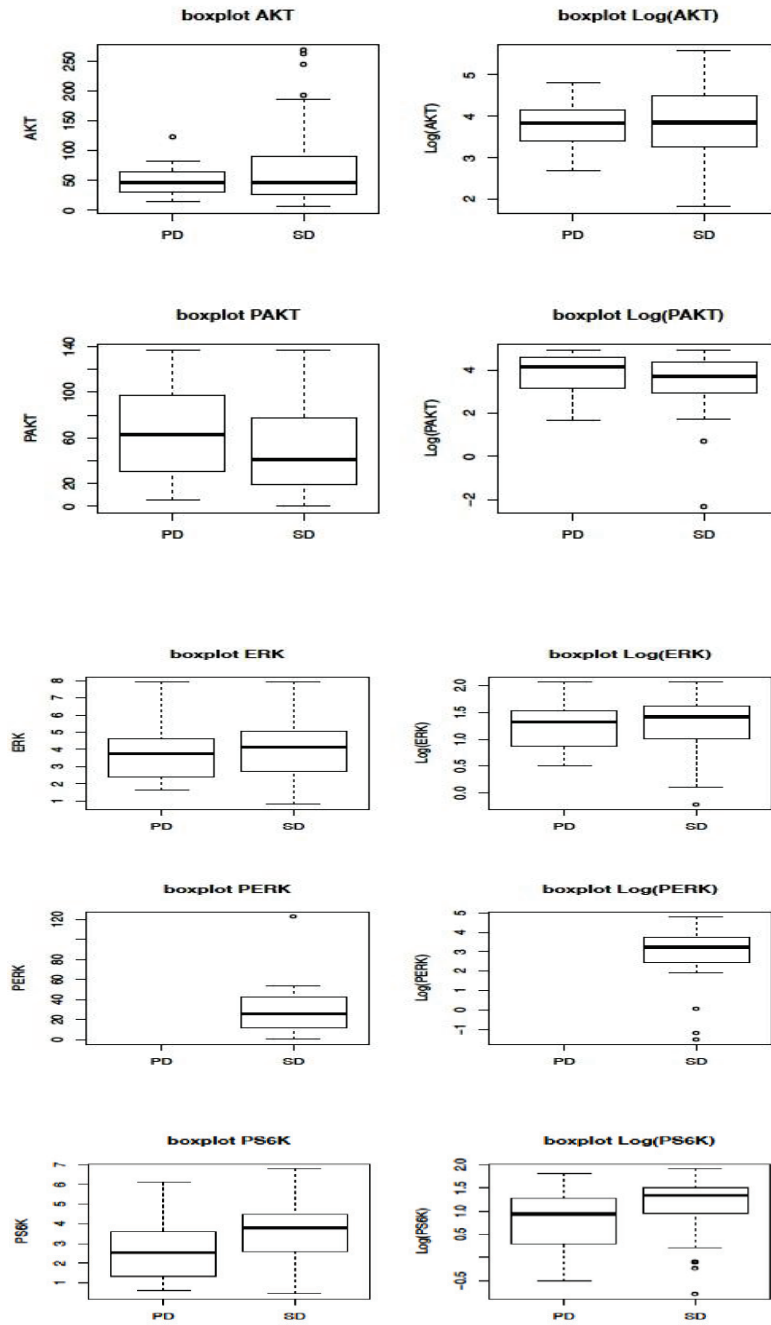


Figure (25): Boxplot correlating tumor biomarker concentrations and clinical response.



**Table (25): Correlation between biomarker slope and clinical response**

<b>Biomarker slope</b>	<b>Progressive disease (Median values)</b>	<b>Stable disease (Median values)</b>	<b>Wilcoxon (p-value)</b>
VEGF	3.945	4.079	0.6331
VEGFR1	-2.424	0.4419	0.5549
VEGFR2	-0.439	-0.038	0.1433
ERK	-0.117	-0.0286	0.1091
AKT	-1.416	-0.0448	1
PAKT	0.6852	0.0085	0.776
PS6K	-0.0321	-0.0109	0.775

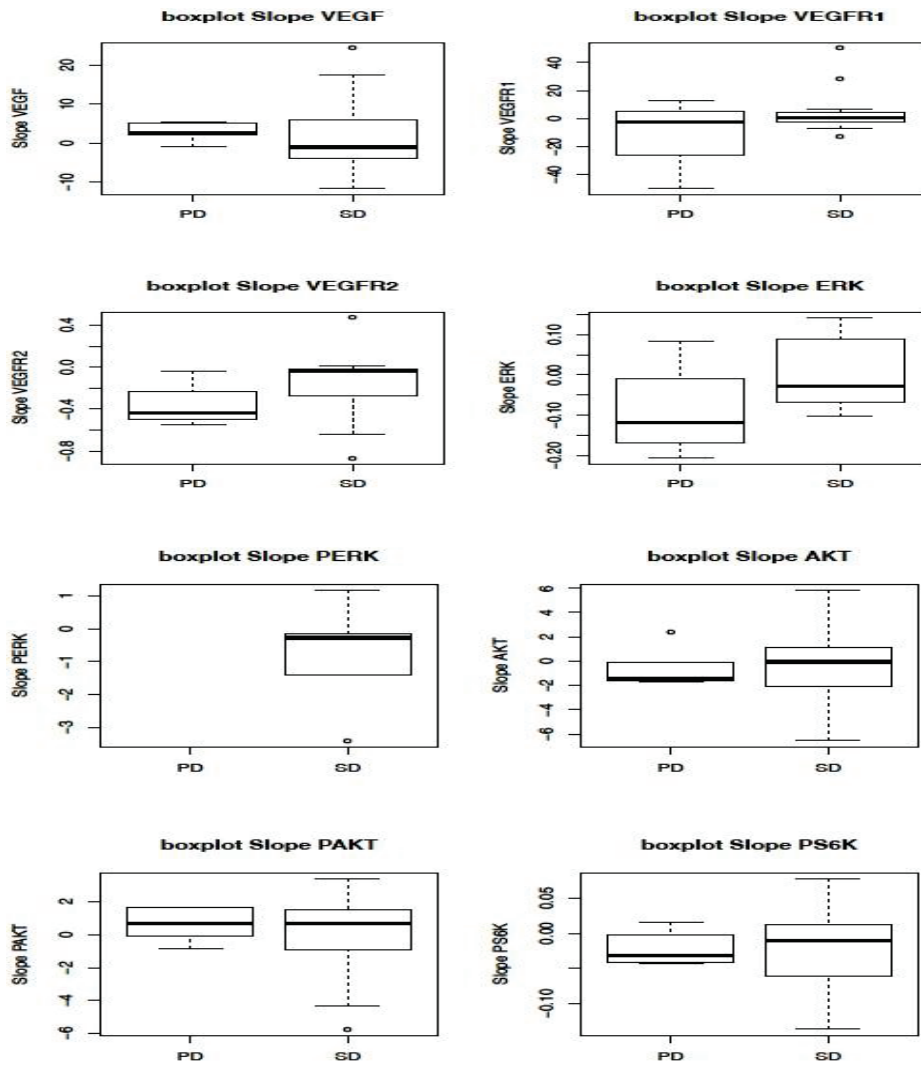


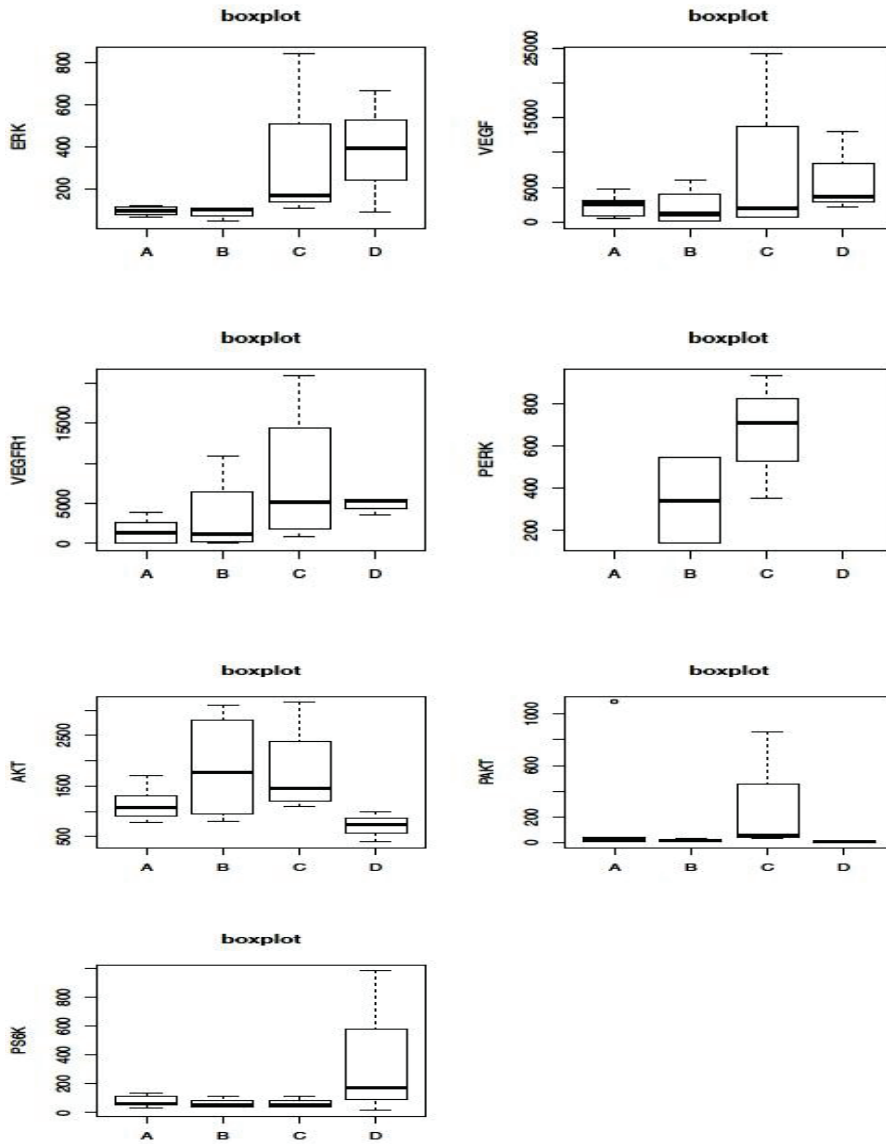
Figure (26): Boxplot correlating tumor biomarker slope and clinical response

### Correlation between Area under the curve and slope of tumor biomarkers and different administration schedules

We compared the effects of different administration schedules A, B, C and D on the median values of AUC and median values of slopes for all tested tumor biomarkers using Kruskal Wallis test that confirmed the absence of any statistical significance ( $p < 0.05$ ) between the four administration schedules corresponding to concentration versus time area under the curve and slopes of tumor biomarkers.

**Table (26): Comparison between different administration schedules and area under the curve of tumor biomarkers**

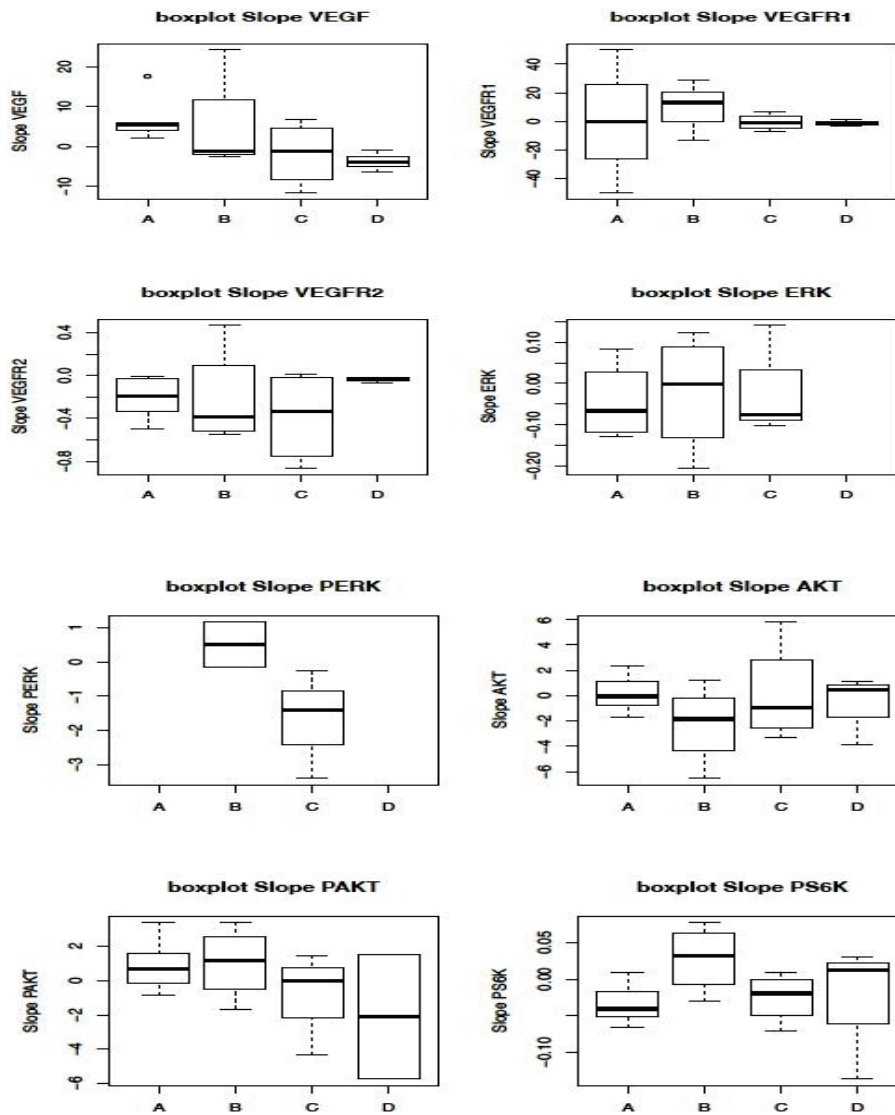
AUC Median value	Bras A	Bras B	Bras C	Bras D	Kruskal Wallis	P values
VEGF	2610.1	1181.05	1996.7	3700.6	2.85	0.4153
VEGFR1	1283.5	1179	5203	5325	5.1432	0.1616
VEGFR2	301	205.5	231	64.2	0.9503	0.8133
ERK	NA	99.85	NA	393.9	4.9762	0.1735
AKT	1079.5	1777.4	1454	741.4	6.7799	0.07926
PAKT	29.3	17.5	NA	NA	4.1071	0.2501
PS6K	62.2	51.35	51.25	172.7	1.391	0.7077



**Figure (27):** Boxplot comparing different administration schedules and area under the curve of tumor biomarkers

**Table (27): Comparison between different administration schedules and slopes of tumor biomarkers.**

Médiane des pentes	Bras A	Bras B	Bras C	Bras D	Kruskal wallis	P values
VEGF	0.109	0.0368	-0.0317	-0.0215	6.3889	0.09415
VEGFR1	0.0371	-0.0257	0.0309	0.0309	0.5619	0.9051
VEGFR2		-2.724	-2.16	-17.72	1.4114	0.7029
ERK	0.669	3.406	7.543	6.681	0.3485	0.8401
pERK	0.102	-0.0306	-0.223	-0.1007	3	0.08326
AKT	0.83	-0.136	0.241	-0.148	2.1972	0.5325
pAKT	-0.271	-1.3204	-0.478	2.665	1.5479	0.671
PS6K	-13.61	7.142	5.563	-14.498	5.3363	0.1488



**Figure (28):** Boxplot comparing different administration schedules and slopes of tumor biomarkers.

### Results of the analysis of the literature review

Identification of the OBD was the primary endpoint in the trials for 11 drugs (34.4%), whilst MTD was the primary endpoint of the 21 remaining drugs (65.6%). For 26 of the 32 studied agents, no MTD were reported (81.2%). Among the 6 drugs with reported MTD, the OBDs were consistently lower than the MTDs (median 12.5%, range 7.5% to 15%). Dose escalations were performed up to OBDs, and not to MTDs, for 3 drugs during phase I trials. Of the 32 drugs with reported OBDs, 15 (46.8%) were investigated in registration phase III trials: Trial outcomes were considered positive for 12 of the 15 drugs (80.0%) tested at OBDs, and negative for two drugs tested at OBD (13.3%) and one drug tested at a different dose from OBD (6.66 %) (brivanib). In addition to these drugs, 4 drugs (dasatinib, pembrolizumab, venetoclax and avelumab) were granted accelerated FDA approvals based on promising ORRs during phase II trials.

## Discussion

Consistent with data from previous phase I trials <sup>134</sup>, the preliminary efficacy outcomes in EVESOR trial confirm that everolimus and sorafenib combination would exhibit promising anti-cancer activity in patients with solid tumors. This was demonstrated in our study as baseline reduction of tumor size especially for patients with gynaecological adenocarcinomas and cholangiocarcinomas. These patients were included in all administration schedules A, B, C and D at dose level 1 (everolimus 5 mg qid and sorafenib 200 mg bid). In addition, the combination regimen of everolimus and sorafenib was generally well tolerated by all patients with advanced solid tumors included in the study.

Despite hints of interesting activity in initial trials <sup>138-140</sup>, as acknowledged by drug companies, the development of this combination was stopped, due to an excessively high toxicity index. However, the uniform design selected by most investigators to date is disputable. Those early phase trials have relied on continuous administration of both drugs, and they used the same dosing schedules as those used as single agents. Indeed, no potential PK and/or PD interactions between the medicines, or no alternative dosing schedules for the two drugs were considered. Before abandoning such a promising combined drug approach, we and others have considered of interest exploration of interactions and alternative doses / dosing schedules, with the aim of maximizing the benefit/toxicity ratio <sup>144</sup>.



EVESOR trial was designed to address the lack of a benefit/toxicity ratio for this drug combination. We reasoned that mathematical modeling may be able to define the optimal doses and dosing schedules, based on relevant data provided by an adequately planned multi-parameter trial . As expected, the two intermittent dosing schedules tested in this trial (Arm C: everolimus and sorafenib alternating every weeks, and arm D: continuous dosing of everolimus and sorafenib 3 days-on 4 days-off) were better tolerated than the other schedules. Interestingly, these preliminary efficacy outcomes suggested that intermittent dosing schedules may be at least as effective, if not more effective, than continuous schedules. This finding may be explained by the lower frequency of required dose reductions in intermittent arms compared to continuous arms.

The doses and dosing schedules of the respective drugs that compose anti-angiogenic-containing combinations might have a strong impact on treatment effects<sup>163</sup>.

Similar to our findings, Bagri et al.,2010 compared the efficacy of 2 durations of treatment with an anti-VEGF monoclonal antibody: 3 week treatment versus continuous treatment<sup>164</sup>. Other research results suggested that time to event (tumor growth inhibition) was longer in mice that received continuous treatment. Moreover they showed the activity of antiangiogenic drug and chemotherapy combination was dependent on drug administration schedule.<sup>165</sup>

Contradictory results have been reported on effects of short term versus continuous administration of anti-angiogenic drugs. In xenografted mice, Ebos et al.,2009 showed short term treatment with sunitinib given at high dose (120 mg/kg/day for 7 days) could decrease overall survival in

comparison with control. However they reported short term treatment was as effective as continuous treatment (60 mg/kg/day) to delay tumor growth in orthotopic primary tumor models <sup>166</sup>.

Also, Paez-Ribes et al.,2009 showed that antiangiogenic effect of 1 week treatment with an anti-VEGFR2, or with sunitinib, was exacerbated when these anti-angiogenic treatments were given over a longer period of time (4 weeks) <sup>167</sup>.

In the present analysis, we used population approach to assess the pharmacokinetics of sorafenib and everolimus. The outcomes about everolimus pharmacokinetics were in line with the literature data. Moes et al. studied the combination of ciclosporin and everolimus. In that study, a two-compartment pharmacokinetic model with lag-time describing the concentration-time profile of oral everolimus in renal transplant patients has been developed using pharmacokinetic modelling. The results showed that Ideal Body Weight (IBW) significantly contributes to the pharmacokinetics of everolimus by explaining variability in apparent volume of distribution, estimated to be  $(V1/F) = 148$  L. This study was performed with patients on a ciclosporin-free regimen and therefore there is no interaction on everolimus pharmacokinetics <sup>154</sup>.

In line with our findings, CL/F was found to be  $9.94+3.21$  L/h in Japanese patients with advanced solid tumors taking everolimus 5 mg/day. Everolimus was absorbed rapidly, with the C<sub>max</sub> being achieved as early as 1–2 h after oral administration. <sup>168</sup> A recent Phase I study of everolimus performed in Europe and the USA showed that the mean (+SD) C<sub>max</sub> in patients with advanced cancer was 32 (+9) ng/ml at daily doses of 5 mg,

with a mean AUCt of 238 (+77) ng h/ ml. <sup>169</sup>These results for Caucasian patients are similar to those obtained here with Japanese patients. These results are in concordance with our findings as C<sub>max</sub> was found to be achieved at 12.4 ng/ml (0–39.30) within 1.12 hours after oral administration of everolimus 5mg/day. Given the small number of patients in our study, these results suggest that there are no substantial differences in the pharmacokinetics of everolimus between the two populations.

Regarding sorafenib, the estimated typical values of apparent clearance and V<sub>d</sub> were 5 L/h and 231 L respectively, which slightly differ from Rajagopalan et al.,2007 who reported 3.31 L/h and 110 L respectively <sup>170</sup>. Similarly, Lokesh et al.,2011 studied sorafenib PK that were assessed in 111 patients enrolled in five phase I and II clinical trials, where sorafenib 200 or 400 mg was administered twice daily as a single agent or in combination therapy. PK model parameter estimates (range) for an 80 kg patient were clearance 8.13 l h<sup>-1</sup> (3.6–22.3 l h<sup>-1</sup>), volume 213 l (50–1000 l), mean absorption transit time 1.98 h (0.5–13 h)<sup>171</sup>.

These discrepancies might be explained by (i) the large inter-individual variability in PK parameters, resulting in a sampling fluctuation of the population parameters estimated from a small number of patients, (ii) differences in the sampling design, (iii) variation of the fraction of dose absorbed (F), because sorafenib is a drug with low absorption due to poor solubility in gastro-intestinal fluids. With this kind of drug, food quantity and content may have a wide influence on drug absorption.

No significant PK interactions between everolimus and sorafenib were identified in our trial, although we observed that addition of sorafenib

to everolimus led to everolimus reduced maximal concentration by 24.2%. Similarly, everolimus maximal concentration was reduced by 30% when combined to sorafenib in a phase I trial SORAVE with 19 solid tumor patients. <sup>147,172 173</sup>

However, it was shown in previous trials that addition of everolimus to sorafenib had no impact on sorafenib C<sub>max</sub> <sup>142,174</sup>. No PK interactions between everolimus and sorafenib were reported in phase I trial conducted by Toffolario et al., 2014<sup>134</sup>. This phase I study demonstrated that everolimus and sorafenib can be safely associated at daily doses of 2.5 mg of everolimus and 600 mg of sorafenib as no modification of the PK profile of everolimus in association with sorafenib were observed <sup>134</sup>.

Furthermore, Harzstark et al.,2011 showed the absence of pharmacokinetic interaction of sequential cohorts of patients received escalating doses of everolimus and sorafenib combination in 28-day cycles <sup>142</sup>.

In our opinion, although it is difficult to have a clear conclusion about PK interactions between everolimus and sorafenib, we assume the risk of clinically significant PK drug interactions is low.

No relationship was found between PK parameters and toxicity.

Although, we noted a higher risk of adverse events in continuous arms A and B than in intermittent arms C and D, no correlations between the different administration schedules and adverse events of all grades were found, nor were between calculated pharmacokinetic parameters of exposure (AUC and C<sub>max</sub>) of both drugs and toxicities. Our study suggests

that changing dosing regimen of the two combined drugs according to administration schedules A, B, C and D had no impact on the outcomes of overall toxicities.

This finding is in concordance with some previous studies demonstrating that changing dose regimen of either sorafenib or everolimus to continuous versus non-continuous or daily versus weekly doses at different dose levels had no impact on toxicity as drug-related toxicities were mostly mild to moderate in severity and unrelated to the dosing schedule<sup>175</sup>.

In contrary to our findings, other studies showed that dosing schedules and doses of sorafenib might impact on pharmacokinetic and pharmacodynamic effects of the drug combination. Fukuda et al., 2013 demonstrated that the incidence of adverse events is related to serum concentration of sorafenib in Japanese patients with hepatocellular carcinoma and renal cell carcinoma. that were significantly greater in patients with grade  $\geq 2$  hand foot syndrome and hypertension than in those not experiencing the adverse events ( $p = 0.0045$  and  $0.0453$ , respectively). Another study by Hénin et al., 2013 showed the effect of dose fractionation of sorafenib and the outcome of hand foot syndrome adverse event using pharmacokinetics-pharmacodynamics (PK-PD) modeling<sup>176,177</sup>. These contradictory findings (the lack of correlation between exposure and toxicity in our study) might be explained by a lack of power due to the large variability of sorafenib exposure.

Interestingly, preliminary PD outcomes may suggest potential PD interactions between both drugs, in terms of inhibitions of the two PI3K-

AKT-mTor & RAS-RAF-ERK signalling pathways, or anti-angiogenic effects induced by the combination <sup>178</sup>. These interactions which may suggest changes in PD effects of a drug by the addition of another one have still to be confirmed.

Several investigators have suggested that the kinetics of soluble markers of angiogenesis might be predictive of anti-angiogenic drugs efficacy <sup>179-181</sup>. They might be surrogate markers of treatment efficacy. In the present study, we assessed VEGF, VEGFR1 and VEGFR2 as markers of antiangiogenic activity.

We found that serum VEGF concentration were increased by 1.5 to 4 fold after sorafenib addition to everolimus in Arm A while this effect was abolished by a mean of 40.7 % on addition of everolimus to sorafenib in Arm B. On the other hand, everolimus when given alone in Arm A, decreased VEGF serum concentrations. Meanwhile, in intermittent schedules C and D, increased VEGF serum concentrations levels induced by sorafenib were maintained at lower levels in the presence of everolimus.

In concordance with our findings, it was demonstrated by Piguet et al.,2011 who studied the effect of combined everolimus-sorafenib on tumors in vivo, that mRNA levels of VEGF-A were increased by 86% after sorafenib although this effect was blunted to 49% on addition of everolimus. In addition, it was reported that the combination of the two drugs, either in a concomitant or sequential regimen, exerted an antiproliferative and an antiangiogenic effects assessed by inhibition of vessel sprouting <sup>182</sup>. Similarly, the combination of sorafenib and PI-103 (a dual PI3K/mTOR inhibitor) synergistically inhibited EGF stimulated cell proliferation by 61% (p<0.001; n=12). The effect of combination of the two drugs was

significantly different from the inhibitory effect of sorafenib ( $p=0.01$ ) and PI-103 ( $p=0.001$ ).

The upregulation of VEGF-A mRNA in the tumor by sorafenib, an effect also reported with other receptor tyrosine kinase inhibitors such as vatalanib<sup>183</sup> and sunitinib<sup>184</sup>, can be attributed to a feedback response to the suppressed VEGF receptor signaling<sup>185</sup>. Also, the antiangiogenic effect of everolimus explained by Lane and colleagues were due to the combination of a reduced VEGF production in tumor cells and direct action on mTOR signaling in non tumor pericytes and endothelial cells<sup>186</sup>. It was previously reported that another mTOR inhibitor, sirolimus, decreased VEGF-A in MH-3924A–derived tumors under different experimental circumstances. A similar reduction was not detected with everolimus, although the sorafenib-induced increase in VEGF-a mRNA tended to be less acute in the presence of everolimus.<sup>187</sup>

In our trial, we found that sorafenib markedly decreased VEGFR1&VEGFR2 concentrations either when given alone or concomitantly and sequentially with everolimus as in Arm B and Arm D. In line with our results, previous study analysis showed that VEGF-induced phosphorylation of VEGFR2 was strikingly reduced by sorafenib treatment in both SW13 and H295R adrenocortical cancer cells<sup>188</sup>. This might be explained as sorafenib acts on multiple tyrosine kinases including VEGFR2<sup>189,190</sup> and it can directly inhibit tumor cell growth via Raf-MEK-ERK signaling<sup>191</sup>.

As a result, Mariniello et al.,2012<sup>188</sup> demonstrated that combination therapy produced remarkable tumor growth inhibitory effects on both SW13

and H295R xenografts, as shown by the significant reduction of the average bioluminescence of tumors from treated animals compared with the placebo.

Sorafenib when given concomitantly or alternatively with everolimus in Arm A and Arm C, reversed the decrease of VEGFR1&VEGFR2 concentration in blood exerted by everolimus. This might be explained as exposure to rebound effect that could be provoked partly by an increased concentration of growth factors such as VEGF, which may fuel tumor growth if left unopposed <sup>192</sup> or due to an upregulation of VEGF as it might be the case in our study. The upregulation of VEGF serum concentrations by the combination of sorafenib and everolimus, might be supporting the hypothesis that VEGF is upregulated at the transcriptional level in some tumors due to a feedback response to suppressed VEGF receptor signaling <sup>185</sup>.

Several studies have shown that inhibition of the expression of VEGF receptors reduced growth and invasion of bladder cancer cells, similar to what was observed in cells with the depletion of VEGF expression <sup>193,194</sup>. Previous studies found a significant correlation between VEGF and VEGFR1, and VEGFR1 and VEGFR2. It is likely that when co-expressed, the VEGF/VEGFR pathways are activated, and VEGF and its receptors may cooperatively promote proliferation, survival and invasion of tumor cells <sup>195</sup>.

In the present study, we measured inhibition of PI3K-AKT-mTor and RAS-RAF-ERK signaling pathways in PBMCs by assessing expression of ERK; p-ERK, p-70S6K, AKT, p-AKT as well as soluble markers of angiogenesis.



Previous studies showed that inhibition of PI3K-AKT-mTor by everolimus in tumors can be reproducibly predicted by measuring 4EBP1; p-4EBP1; S6K; p-S6K in PBMCs using immunoblotting <sup>100</sup>.

The effects of sorafenib on RAS-RAF-ERK signaling pathway may also be measured in PBMCs. In a phase II trial with 73 patients with ovarian or primary peritoneal carcinomas, inhibition of this pathway could be successfully measured by ERK and p-ERK in PBMCs using immunohistochemistry <sup>196</sup>.

In the present analysis, we found that everolimus alone in Arm A reduced concentrations of p-ERK and p- P70S6K while it had no effect on AKT total and p-AKT. On the other hand, sorafenib monotherapy in Arm B reduced p-ERK, also decreased p-AKT and P70S6K while AKT total, ERK total remain unchanged. The addition of everolimus to sorafenib in Arm B didn't result in any reduction of p- AKT, p- ERK and P70S6K.

In line with previously studied potential mechanism of everolimus, the phosphorylation status of downstream targets of mTOR was investigated and showed a significant reduction in the levels of p-P70S6K, in everolimus treated HCC tumors. There was no impact of everolimus on ERK1/2 and AKT phosphorylation<sup>197</sup>. Moreover, Li Liu et al.<sup>198</sup>, demonstrated that sorafenib inhibits RAF/MEK/ERK signaling pathway in HCC cell lines through inhibited MEK and ERK phosphorylation at a concentration of between 3 and 10 umol/L. Total MEK, ERK, and AKT levels were unchanged, and no changes were observed in the phosphorylation levels of AKT.

In concordance with these findings, Mariniello et al.,<sup>2012</sup> demonstrated that everolimus abolished P70S6K phosphorylation in both SW13 (metastatic) and H295R (primary) adrenocortical carcinoma cell while it only slightly reduced p-AKT at the highest drug concentration. On the other hand, sorafenib (5  $\mu$ M) markedly reduced p-AKT, pERK1/2 and p70S6K in SW13, while only p-ERK seemed to be reduced in H295R cells.<sup>188</sup>

We showed in our study the benefit of combining everolimus, an mTOR inhibitor with sorafenib, an inhibitor of B-RAF and RAF-1 kinases, on antitumor activity. Our findings demonstrated that there was a correlation between clinical response and tumor biomarkers manifested by clear distinction between responders (stable disease more than 4 months) and non-responders, progressive disease related to VEGF and P70S6K biomarkers suggesting that the distinction between responders and non responders may lie in operative signaling pathways regulating tumor cell growth and survival, which supposed to be in our case angiogenesis and mTOR signaling pathway.

In concordance to our results, a previous preclinical study investigating the antiproliferative and antiangiogenic effects of sorafenib and everolimus combination in orthotopic model of hepatocellular carcinoma. ascribed the superior ability of the combination to slow tumor growth to impaired tumor angiogenesis and vascularisation <sup>182</sup>. This effect is in consistence with previous in vitro and in vivo experiments showing that addition of everolimus to sorafenib significantly reduced the tumor size in SW13 and H295R xenograft mice and also led to significant increase in median survival in SW13 models <sup>188</sup> and with those reported that the

combination of sorafenib and rapamycin has shown synergistic inhibition of tumor size in hepatocellular carcinoma xenografts <sup>197</sup>.

.The serum inhibition of the phosphorylation of the ribosomal protein S6 elicited by the combination and translated by reduced concentrations values of P70S6K, confirms the combined effects of pharmacologic inhibition of the mTOR signaling in endothelial cells. In line, the assessment of the effects of sorafenib and everolimus on intracellular pathways in ACC cell lines, reported almost complete inhibition of p-AKT and p70S6K with either sorafenib (5  $\mu$ M) or everolimus (0.1  $\mu$ M).<sup>184,188</sup>.

The preliminary efficacy outcomes in EVESOR trial may confirm that everolimus and sorafenib combination exhibits promising anti-cancer activity in patients with solid tumors, as confirmed by overall response rates (ORR): PR 11%; SD 78%. This was demonstrated by the waterfall plot as best overall change from baseline in target lesion measurements for all studied solid tumors the combination therapy of everolimus and sorafenib demonstrated promising results mainly in gynaecological adenocarcinomas (endometrial, fallopian and cervical adenocarcinomas) and cholangiocarcinoma followed by breast adenocarcinomas and anus squamous cell carcinoma. On the other hand, hepatocellular carcinomas and colon adenocarcinomas showed a progression on the combination.

The mechanism of clinical responsiveness of endometrial tumors to everolimus and sorafenib combination could be explained as follows: dysregulation of phosphatase and tensin homolog (PTEN) and the gene that encodes the PI3K, PIK3CA, are the most common mutations in endometrial carcinoma <sup>102,199</sup>. Loss of PTEN or activation of PIK3CA results in constitutive activation of AKT, which leads to up-regulation of mTOR. As

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a result, there has been a very strong rationale for development of everolimus in endometrial cancers <sup>102</sup>. Preclinical studies demonstrated inhibition of PI3K-AKT-mTor signaling pathway was relevant <sup>102</sup>.

The role of sorafenib has been studied in treatment of endometrial cancer. There are insights for considering RAS-RAF-ERK signaling pathways is frequently dysregulated in endometrial carcinomas cell lines although the mechanisms remain unclear <sup>200</sup>. In a preclinical study, Llovet et al., 2008 showed that sorafenib induces apoptosis of endometrial cancer cell lines and human primary cultures and sensitises these cells to Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL). <sup>201</sup>.

In line with our findings, the efficacy of anti-angiogenic drugs such as sorafenib or bevacizumab in squamous cervical carcinoma has been suggested in on-going clinical trials <sup>202,203</sup>. Activations/mutations of RAS-RAF-ERK signaling pathway have been reported in patients with squamous cervical carcinomas <sup>204 199 199</sup>.

The strong anti-proliferative activity of mTor inhibitors in squamous cell cervical cancer patients has been suggested in some studies. Preliminary data, however, suggest that <sup>205</sup> activation of the mTOR pathway represents a common feature of cervical squamous cell carcinoma. Prior reports described the presence of phosphorylated p70S6 kinase in cervical squamous cell carcinoma <sup>206</sup>, although pS6 seems to be a more reliable marker to document TORC1 activation by IHC than its upstream kinase p70S6K <sup>207</sup>.

Encouraging results confirming our findings suggest that in cholangiocarcinoma, as well as in normal cholangiocytes, bile acids activate the two main signaling pathways (Ras/Raf/MAPK and the PI3K/Akt/mTOR) via a TGF- $\alpha$ -dependent mechanism. Bile acid mitogenesis may facilitate the progression of cholangiocarcinoma and blocking the TGF- $\alpha$ /EGFR autocrine pathway attenuates bile acid-stimulated growth of cholangiocarcinoma cell lines. On these bases, several lines of evidence may point to the usefulness of EGFR targeting as an adjuvant therapy in cholangiocarcinoma <sup>208,209</sup>

The mechanism by which sorafenib with everolimus exerted their effects could be attributed to antiangiogenic action of sorafenib in patients with advanced breast cancer. Indeed, angiogenesis is largely implicated in pathogenesis of malignancy and in metastases of breast cancers <sup>210, 211</sup>. Moreover the RAS-RAF-ERK pathway is involved in proliferation of breast cancer cells <sup>211</sup>.

Inhibition of PI3K-AKT-mTor signaling pathway might enable to overcome resistance to anti-HER2 treatments in HER-2 positive breast cancers <sup>102,212, 213</sup>.

Breast cancer investigators concluded that sorafenib development should be considered in combination with other anti-cancer agents. It was tested in association with chemotherapies such as capecitabine and paclitaxel with promising results <sup>214</sup>.

The potential PD efficacy of everolimus given as a neoadjuvant treatment for 14 days before surgery (5mg/day) was suggested in a phase 2 trial with 31 breast cancer patients. Everolimus treatment significantly

decreased proliferation, particularly in HER-2 positive tumors. Nuclear expression of p-AKT was significantly reduced with treatment. Tumors exhibited a significant reduction in cytoplasmic p-AKT. p-S6 staining was significantly reduced <sup>215</sup>.

In contrast to our findings, promising anti-cancer activity has been suggested for sorafenib in association with irinotecan or cetuximab in metastatic colorectal cancer patients in several phase 1 and 2 trials <sup>216,217</sup>.

<sup>218</sup>.

Furthermore, our results showed a progressive disease in patients with hepatocellular carcinoma, treated with the combination of everolimus and sorafenib, in contrary to previous findings of a preclinical study. After hepatic implantation of Morris Hepatoma (MH) cells, rats were randomly allocated to everolimus (5 mg/kg, 2×/week), sorafenib (7.5 mg/kg/d), combined everolimus and sorafenib, sequential sorafenib (2 weeks) then everolimus (3 weeks), or control groups. Combined treatment with everolimus and sorafenib exerted a stronger antitumoral effect on tumors than monotherapy. Everolimus retained antitumoral properties when administered sequentially after sorafenib <sup>130</sup>.

This discrepancy could be explained by the administration schedule to which patients of our study were allocated. They were treated on administration schedule A where sorafenib is administered in combination with everolimus after two weeks run-on period of everolimus. This sequence is different from that given in previous studies.

Although PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways are frequently deregulated in pancreatic cancer cells, phase 1 and 2 trials of everolimus and sorafenib showed poor anti-cancer activities of these drugs when given as single agents. It is considered that both agents should be tested in combination.

Our findings suggested that combination of everolimus and sorafenib induced a stable disease in patients with pancreatic adenocarcinomas. In line with our findings, in a phase 2 trial, 33 patients with gemcitabine-refractory, metastatic pancreatic cancers were treated continuously with everolimus at 10 mg daily. No complete or partial treatment responses were noted, and only seven patients (21%) had stable disease.<sup>219</sup> Another phase 2 trial showed poor activity of everolimus in patients with advanced pancreatic cancers<sup>220,221</sup>.

The recommended dose for phase II studies, or the maximum tolerated dose, cannot be determined yet, as dose escalations in intermittent schedules are still ongoing. The model & simulation-based optimal doses/dosing schedules cannot be defined yet, as well. As a consequence, the outcomes of the present study should be considered with caution. The limited numbers of patients in each arm largely reduce the power of the hypotheses and outcomes set in the present article. We should wait for the results of PD analyses and dynamic imaging tests to get more understanding of the impacts of either drug doses and dosing schedules on the combination benefit/toxicity ratio.

Despite these limitations, the present intermediary outcomes confirm the feasibility of EVESOR trial. The preliminary PK and clinical results support the lack of PK interactions between both drugs and potential better toxicity and efficacy profiles in intermittent arms. These assumptions will however have to be confirmed with further outcomes. Moreover, the optimized doses/dosing schedules proposed by the model will have subsequently to be tested. The innovative design and assumptions of EVESOR trial might contribute to address some unanswered issues about the drug development of this abandoned targeted combination, as well as of other associations <sup>222</sup>.

On the other hand, The optimal biologically effective dose (OBD) is frequently described as a promising and rational strategy for drug development, but the relevance in terms of clinical efficacy is still unknown<sup>223,224</sup>. If OBD defined during phase I trials was found to be clinically effective, it would be interesting for drug development as it would probably reduce toxicity and make subsequent development easier, as suggested by Roda et al.,2016<sup>225</sup> Indeed, as found in the present review, OBD is frequently lower than MTD, thereby being less toxic.<sup>224</sup>If we consider that 200 to 250 phase I trials of experimental oncology drugs are published every year <sup>223</sup>, the present study first suggests that OBD is rarely assessed in current early phase I trials. However, it may be a relevant strategy, since 83.3 % of the final approved doses, when the drugs are eventually approved, are consistent with the OBD. This percentage is much higher than the 58% reported with MTD by Fontes Jardim et al. <sup>226</sup>, thereby confirming the assumption done by many experts that MTD is not appropriate for defining the RP2D of molecular targeted agents<sup>225</sup>. Of note,



a drug, namely brivanib, did not receive approval after a trial failing to show non-inferiority compared to sorafenib for hepatocellular carcinoma, when brivanib was used at MTD and not at OBD.

## **Summary and Conclusion**

### ***I- EVESOR study***

#### ***Rationale of EVESOR study***

- The development of everolimus and sorafenib combination was stopped by drug companies, due to the high toxicity index and the lack of a clear benefit/toxicity ratio to guide dose recommendations for a phase II trial. Daily monotherapy regimens of both drugs were used. However, sorafenib dosing schedule may impact on everolimus tumor delivery and thus on toxicity & efficacy. It should be possible to determine the optimized doses and dosing schedules of both drugs, which are able to maximize the benefit/toxicity ratio, using modeling and simulation studies. We designed the first multi-parameter phase I study (EVESOR), based on mathematical modeling of data provided from an adequately designed trial with this objective.

#### ***Design of EVESOR trial***

- EVESOR trial was a four-arm, multiparameter Phase I trial of everolimus and sorafenib. This is an open-label, phase Ib trial where patients with metastatic or locally advanced cancers who are deemed eligible were treated with the combination of sorafenib and everolimus.
- The four schedules were presented as follows: In schedules A and B, respectively, either everolimus or sorafenib will be given alone during a 2-week run-in period before starting continuous administration of the combination (once a day [q.d.] for everolimus; twice a day [b.i.d.] for sorafenib) to assess the ability of each drug to affect the concentrations and PD parameters of the other drug. In schedule C, sorafenib will be

given b.i.d. for a week alternating with q.d. everolimus every other week. In schedule D, sorafenib will be given b.i.d. for 3 days-on 4 days-off, while everolimus will be administered q.d. on a continuous basis.

#### *Assessments of EVESOR study*

##### PK assessments

The PK profiles of sorafenib and everolimus were modeled independently. The structural model for sorafenib was a 1-compartment model with first order absorption; the structural model for everolimus was a 2-compartment model with first order absorption.

##### PD assessments

We serially measured inhibition of PI3K–AKT–mTor and RAS–RAF–ERK signaling pathways in peripheral blood mononuclear cells (PBMCs), by assessing expression of AKT total, pAKT, total S6K, pS6K, ERK total, pERK using ELISA kits from Invitrogen. Antiangiogenic markers VEGF, VEGFR1, VEGFR2 were measured serially in serum (during cycle one and two and at the same times as PBMCs) using the human ELISA kits from Abcam.

#### **Summary of preliminary results of EVESOR study**

- The preliminary efficacy outcomes in this EVESOR trial indicated that everolimus and sorafenib combination exhibited promising anti-cancer activity in patients with solid tumors, as shown by the overall response rates (ORRs) : partial response : 11% ; stable disease : 78%.

- Everolimus and sorafenib combination showed antitumor activity especially for patients with gynaecological adenocarcinomas and cholangiocarcinomas. These patients were included in administration schedules B, C and D all at dose level 1. (everolimus 5 mg qid and sorafenib 200 mg bid).
- The two tested intermittent dosing schedules tested were better tolerated and showed at least comparable, if not better, efficacy compared to continuous schedules. (ORR in intermittent schedules: PR 20% and SD 80% vs. ORR in continuous schedules: PR 25% and SD 75%)
- The preliminary efficacy outcomes suggest that intermittent dosing schedules may not be less effective at least, if not better, than continuous schedules.
- No clear relationships between PK parameters and toxicity were observed, which suggests that there were no significant PK interactions between everolimus and sorafenib. It may be a ‘proof of concept’ of model-based early-phase trials of targeted agent combinations.
- No relationships between PK parameters and toxicity of efficacy were found. Although, we noted a higher risk of adverse events in continuous arms A and B than in intermittent arms C and D, no correlations between the different administration schedules and adverse events of all grades were found, nor were between calculated pharmacokinetic parameters (AUC and C<sub>max</sub>) of both drugs and toxicities.
- Our study suggests that changing dosing regimen of the two combined drugs according to administration schedules A, B, C and D had no impact on the outcomes of overall toxicities.

- Preliminary PD outcomes may suggest potential PD interactions between both drugs, in terms of inhibitions of the two PI3K-AKT-mTor & RAS-RAF-ARK signalling pathways, or anti-angiogenic effects induced by the combination as previously show

### **Conclusion**

When everolimus was given concurrently with sorafenib on a daily basis, stable disease and response rates were comprised in between 30% to 76% and 5% to 100% respectively. These outcomes suggest dual inhibition of PI3K-AKT-mTor and RAS-RAF-ERK signaling pathway is associated with increased anti-tumor activity. Moreover, there are elements for considering sorafenib might alter delivery of everolimus in tumor site. There is no data about efficacy of intermittent administration of everolimus or sorafenib. We selected tumor sites which may be sensitive to everolimus and sorafenib.

### **II- Data Analysis of the literature review**

An extensive research analysis of the literature review was conducted. to identify all publications of early phase trials defining an OBD for molecular targeted therapies in oncology between 2000 and 2016. The publications of subsequent phases II and III clinical trials of involved drugs were reviewed, along with potential approvals, to compare approved doses to OBDs identified earlier. A final FDA approval was found for 56.2 % drugs with defined OBD. The approved doses were consistent with the reported OBD for 83.3 % drugs. There were exception for 3 drugs. The analysis suggests that, despite being rarely investigated, OBD may be a relevant endpoint for early phase trials, as

it was found to be consistent with subsequent dose approved by FDA, in 83.3 % cases.

### **Publications**

1. **El-Madani M, Colomban O, Tod M, Maillet D, Peron J, Rodriguez-Lafrasse C, Badary O, Valette PJ, Lefort T, Cassier P, El-Shenawy SM, EL-Demerdash E, Hommel-Fontaine J, Guitton J, Gagnieu MC, Ibrahim BM, Barrois C, Freyer G & You B** : « EVESOR, the first model-based multi-parameter academic phase 1 trial meant to optimize the benefit/toxicity ratio of everolimus (EVE) & sorafenib (SOR) combination: initial results”. *Future Oncol.* (2017) 13(8), 679–693
2. **You B, El Madani M, Henin E, Tod M, Maillet D, Peron J, & Hommel-Fontaine J** : “EVESOR, the first model-based multi-parameter academic phase 1 trial meant to optimize the benefit/toxicity ratio of everolimus (EVE) & sorafenib (SOR) combination: initial results.” 2016 ASCO annual meeting abstract #166035
3. **El-Madani M, Hénin E, Lefort T, Tod M, Freyer G, Cassier P, Valette PJ, Rodriguez-Lafrasse C, Berger F, Guitton J, Lachuer J, Slimane K, Barrois C & You B** : « Multiparameter Phase I trials: a tool for model-based development of

targeted agent combinations – example of EVESOR trial »  
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4. **Mohamed MA, Henin E, Freyer G, Tod M, Rodriguez-Lafrasse C, Valette P, & You B.** «EVESOR the first model-based multi-parameter phase 1 trial meant to optimize the benefit/toxicity ratio of everolimus and sorafenib association: preliminary PD outcomes.» 2014 ESMO annual meeting abstract 465
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