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Clinical Trial Results

Selection of Protein Kinase Inhibitors Based on Tumor Tissue Kinase Activity Profiles in Patients with Refractory Solid Malignancies: An Interventional Molecular Profiling Study

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Trial Information

- **ClinicalTrials.gov Identifier**: NCT01190241
- **Sponsor(s)**: Vitromics Healthcare Services
- **Principal Investigator**: Henk M.W. Verheul
- **IRB Approved**: Yes

Lessons Learned

- Clinically applicable tools are needed for treatment selection and repurposing of available protein kinase inhibitors (PKIs) in patients with advanced solid tumors refractory to standard treatment.
- Using a tyrosine kinase peptide substrate microarray, observed inhibitory activity in vitro could not sufficiently predict clinical benefit of treatment with the selected PKI.

Abstract

Background. This exploratory molecular profiling study determined the feasibility and benefit of the selection of protein kinase inhibitors (PKIs) based on kinase activity profiling in patients with refractory solid malignancies.

Methods. Adult patients with biopsy-accessible refractory solid tumors were eligible. Per patient, the inhibitory potency of sunitinib, dasatinib, erlotinib, sorafenib, everolimus, and lapatinib was determined in tumor lysates from fresh biopsies using a tyrosine kinase peptide substrate microarray. The most active PKI in this in vitro assay was selected for treatment.

Results. Thirteen patients were enrolled in the feasibility part and underwent tumor biopsy. Of 12 patients in whom kinase activity profiling was performed, 11 started treatment with a selected PKI: dasatinib in 8, sunitinib in 2, and erlotinib in 1 patient(s). Eight patients were evaluable for response. One patient had stable disease (SD) >4 months on sunitinib; one patient had SD at 6 weeks but progressive disease (PD) at 12 weeks. The remaining patients had PD after 6 weeks of treatment.

Conclusion. Kinase inhibition profiles of multiple PKIs can be reliably determined using fresh tumor biopsies from patients with refractory solid tumors. However, the current in vitro microarray selection approach insufficiently predicted clinical benefit of PKI treatment in these patients. ***The Oncologist*** 2018;23:1135–e118

Discussion

Compared with the increased availability of molecular targeted therapies, including numerous protein kinase inhibitors (PKIs), the development of clinical tests to identify patient subgroups most likely to benefit from these therapies is lagging behind. A particular clinical need exists for tools to enable selection of multitargeted PKIs and for patients with advanced solid tumors refractory to standard treatment, who could benefit from repurposing of available drugs. Several potentially useful tumor-profiling platforms such as peptide and (reverse phase) protein microarrays have been suggested to infer kinase activity for treatment stratification or target identification [1–6]. Preclinical and clinical data have shown some indications that a 144-tyrosine kinase peptide substrate microarray may be of value for treatment selection [7, 8]. Hypothesizing that microarray-based kinase activity profiling may be a potential clinical tool for PKI treatment selection in patients refractory to

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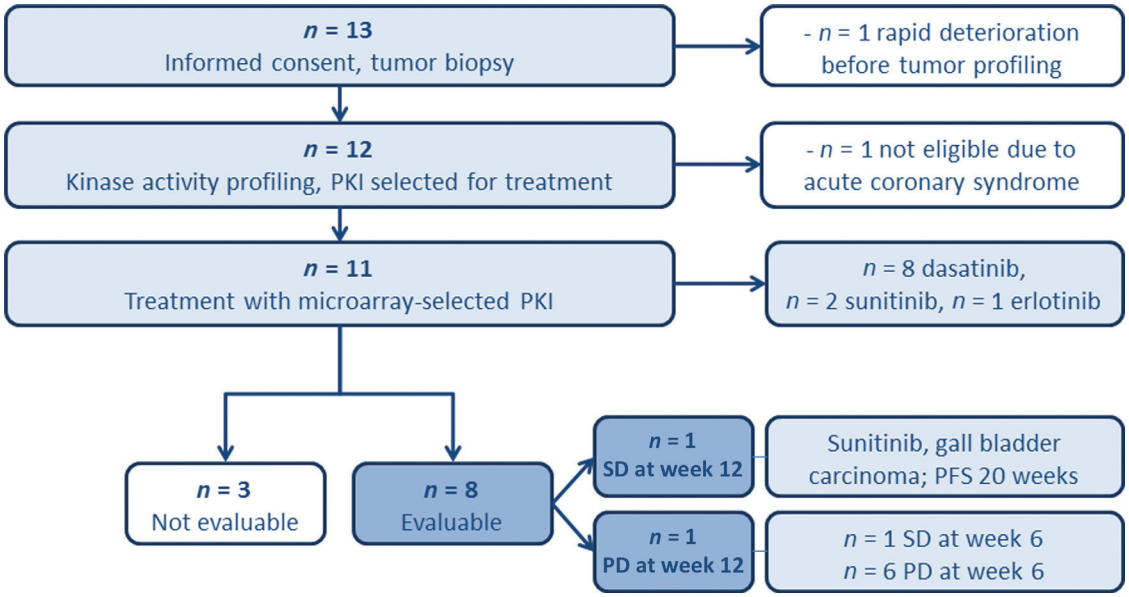

Figure 1. Two of thirteen patients who gave informed consent could not start treatment; one patient progressed rapidly before completion of tumor profiling, and one patient became ineligible after profiling. Three patients for whom dasatinib was selected were not evaluable for response due to early clinical progression ($n = 2$) and patient's refusal of selected treatment ($n = 1$).

Abbreviations: PD, progressive disease; PFS, progression-free survival; PKI, protein kinase inhibitor; SD, stable disease.

standard treatment, we performed a molecular profiling study to select treatment with a registered PKI by in vitro assessment of their inhibitory effect on kinase activity profiles using lysates obtained from fresh tumor biopsy.

Adult patients with biopsy-accessible disease of an advanced solid malignancy, for whom no standard treatment was available, were eligible. Sunitinib, dasatinib, sorafenib, erlotinib, lapatinib, and everolimus were analyzed for their inhibition of kinase activity. Extrapolation of in vitro inhibitory potency to their presumed clinical activity was based on an algorithm considering, per drug, the number of significantly inhibited peptides and percentage of inhibition. The most potent PKI in this assay was then selected for treatment.

Thirteen patients were enrolled. Kinase activity profiling was performed for 12 patients; dasatinib was selected for 9 patients, sunitinib for 2 patients, and erlotinib for 1 patient. Eight of eleven patients who subsequently initiated the selected treatment were evaluable for response. One patient with biliary tract cancer had stable disease (SD) on sunitinib for more than 4 months. One patient treated with dasatinib showed SD at 6 weeks but progressive disease (PD) at 12 weeks; the remaining patients had PD at first evaluation (Fig. 1). Based on our findings after 11 treated patients that dasatinib was selected in 75% of patients but resulted in clinical benefit in $<10\%$ of patients within 6 weeks, we concluded by statistical means that the chance for the trial to succeed to stage II according to prespecified criteria was $<1\%$. The trial was therefore prematurely halted.

In conclusion, we here show that PKI inhibition profiles can be reliably determined using needle biopsies from patients with refractory solid tumors. However, the microarray-based selection strategy was insufficient in predicting clinical benefit upon treatment with the selected PKI. We anticipate that the concentrations used in this in vitro assay should be re-evaluated based on achieved PKI tumor concentrations in patients.

Trial Information	
Disease	Advanced cancer/solid tumor only
Stage of Disease/Treatment	Metastatic/advanced
Prior Therapy	No designated number of regimens
Type of Study – 1	Phase II
Type of Study – 2	Interventional molecular profiling study
Primary Endpoint	Feasibility and clinical benefit rate (CBR), defined as the partial response (PR), complete response (CR), and SD rate after 12 weeks, of treatment selection by tumor kinase activity profiling
Secondary Endpoint	Progression-free survival (PFS) ratio (PFS_2/PFS_1) of a PKI selected by kinase activity profiling (PFS2) compared with the PFS of the treatment regimen on which the patient progressed prior to study inclusion (PFS1)

Additional Details of Endpoints or Study Design
Based on the CBR of approximately 10% observed in phase I studies of the registered PKIs used in this study, we hypothesized that the microarray-based selection would increase the CBR of PKI treatment in this patient population refractory to standard treatment from 10% to 25%. Based on Simon's two-stage optimal trial design, it was calculated that 43 patients (18 in stage I and 25 in stage II) needed to be treated. The interim analysis after stage I required demonstrated clinical benefit for at least 3 of 18 patients to proceed to stage II, in which 25 more patients would be included. If at least 8 of the total 43 patients would demonstrate clinical benefit, the null hypothesis that this treatment selection strategy is only effective in 10% of patients would be rejected. If in stage I $\leq$2 of the 18 patients would have clinical benefit at 12 weeks of treatment, the study would be terminated due to lack of acceptable clinical benefit (efficacy) of this approach. Based on these considerations, this trial was calculated to have 80% power to detect CBR of 25% versus the null rate of 10% with a 5% Type I error rate.

Investigator's Analysis	Level of activity did not meet planned endpoint

Drug Information for Phase II Standard (Treatment Selection) Arm

Drug 1	
Generic/Working Name	Sunitinib
Dose	50 mg per flat dose
Route	p.o.
Schedule of Administration	Once daily
Drug 2	
Generic/Working Name	Erlotinib
Dose	150 mg per flat dose
Route	p.o.
Schedule of Administration	Once daily
Drug 3	
Generic/Working Name	Sorafenib
Dose	400 mg per flat dose
Route	p.o.
Schedule of Administration	Twice daily
Drug 4	
Generic/Working Name	Dasatinib
Dose	70 mg per flat dose
Route	p.o.
Schedule of Administration	Twice daily
Drug 5	
Generic/Working Name	Everolimus
Dose	10 mg per flat dose
Route	p.o.
Schedule of Administration	Once daily
Drug 6	
Generic/Working Name	Lapatinib
Dose	1,250 mg per flat dose
Route	p.o.
Schedule of Administration	Once daily

Patient Characteristics

Number of Patients, Male	7
Number of Patients, Female	5
Stage	IV
Age	Median (range): 62 (26–69)
Number of Prior Systemic Therapies	Median (range): 2 (1–3)
Performance Status: ECOG	0 — 3 1 — 9 2 — 0 3 — 0 Unknown —

Cancer Types or Histologic Subtypes	Colorectal cancer 5 Cholangiocarcinoma 3 Gallbladder cancer 1 Pancreatic cancer 1 Germ cell cancer 1 Synovial sarcoma 1

Primary Assessment Method

Title	Total patient population
Number of Patients Screened	13
Number of Patients Enrolled	12
Number of Patients Evaluable for Toxicity	12
Number of Patients Evaluated for Efficacy	8
Evaluation Method	RECIST 1.0
Response Assessment CR	$n = 0$
Response Assessment PR	$n = 0$
Response Assessment SD	$n = 1$
Response Assessment PD	$n = 7$
(Median) Duration Assessments PFS	6 weeks
Outcome Notes	According to the study design, a stopping rule would apply if <3 of 18 patients in stage I would have clinical benefit at 12 weeks of treatment. However, based on our findings after 11 treated patients that dasatinib was selected in 75% of patients, resulting in clinical benefit in $<10\%$ of patients within 6 weeks, we concluded by statistical means that the chance for the trial to succeed to stage II according to prespecified criteria was $<1\%$. Therefore, the trial was prematurely halted. As a consequence, the secondary objective to determine the PFS_2/PFS_1 ratio of microarray-selected PKI treatment became futile as well.

Adverse Events

All Cycles

Name	NC/NA	1	2	3	4	5	All grades
Thromboembolic event	87%	0%	0%	13%	0%	0%	13%
Fatigue	75%	0%	0%	25%	0%	0%	25%
Noncardiac chest pain	87%	0%	0%	13%	0%	0%	13%

Only grade ≥ 3 adverse events that were potentially related to the study treatment are shown. Treatment with dasatinib caused more significant toxicity compared with sunitinib and erlotinib (Common Terminology Criteria for Adverse Events grade ≥ 3 adverse events in 3/8 vs. 0/3 patients, respectively).
Abbreviation: NC/NA, no change from baseline/no adverse event.

Assessment, Analysis, and Discussion

Completion	Study terminated before completion
Terminated Reason	Did not fully accrue
Investigator's Assessment	Level of activity did not meet planned endpoint

This study represents the first attempt to predict the clinical activity of six approved protein kinase inhibitors (PKIs) in individual patients based on their in vitro activity in lysates from fresh-frozen tumor biopsies, followed by selection of the most active agent for personalized treatment. Adult patients with progressive, measurable, and biopsy-accessible disease of an unresectable and/or metastatic solid malignancy refractory to standard treatment, with Eastern Cooperative Oncology Group performance status of 0–2 were eligible. Tumor needle biopsies were taken with up to three passes under ultrasound- or

computed tomography guidance. Biopsies with ≥50% tumor cells upon hematoxylin and eosin staining were considered representative. Kinase activity profiling was performed using a tyrosine kinase peptide substrate microarray (PamChip) consisting of 144 peptide substrates (PamGene, Hertogenbosch, The Netherlands), including phosphorylation sites for epidermal growth factor receptor, vascular endothelial growth factor receptor, and platelet-derived growth factor receptor (Fig. 2) [9]. Per patient, sunitinib, sorafenib, erlotinib, dasatinib, everolimus, and lapatinib were analyzed in vitro for their inhibition of kinase activity; the most potent PKI in this assay was then selected for treatment (Fig. 3). PKIs were prescribed according to standard dose and schedule. Treatment was continued until disease progression or unacceptable toxicity.

Thirteen patients, of whom five had metastatic colorectal cancer and four biliary tract cancer, were enrolled in the study and underwent tumor needle biopsy (Table 1). Kinase activity profiling was performed for 12 patients; dasatinib was selected for 9 patients, sunitinib for 2 patients, and erlotinib for 1 patient. Eleven patients subsequently initiated the selected treatment after a median of 17 days following tumor biopsy (Fig. 1). Algorithm scores for the selected PKIs are shown in Table 2. Eight of eleven patients who started treatment with the microarray-selected PKI were evaluable for response. One patient reached the endpoint of clinical benefit at 12 weeks of treatment. This patient with gallbladder carcinoma had stable disease >4 months on selected treatment with sunitinib. Of note, this drug has shown a 50% disease control rate in 54 patients with advanced, chemotherapy-refractory biliary tract cancer but with a median duration of disease control of only 2.4 months [10]. Although we showed that this selection strategy was technically and clinically feasible in this patient population, this resulted in a relative selection preference for dasatinib without subsequent evidence of clinical activity in the patients who went on to receive this drug. Therefore, after treatment of 11 patients, the study was halted for lack of drug selection and clinical activity and a calculated probability of less than 1% for the trial to succeed to stage II.

The (aberrant) biological activity of kinases in tumor cells is only one of the main determinants for response to treatment with PKIs. Other major contributing factors are the target specificity and affinity of the PKI for individual kinases that are biologically active and the bioavailability of the drug at the target site, in tumor cells [11, 12]. PKI bioavailability is multifactorially determined by chemical characteristics such as pH and lipophilicity that influence their intestinal uptake after oral ingestion, protein-binding capacity, and ability to cross cell membranes. The latter will determine the circulating free concentration of a specific PKI and thereby its diffusion rate into the tumor microenvironment [13]. The relative contribution of each of these determinants to response and their interplay are difficult to evaluate. We observed a striking disconnection between the potency of dasatinib in the assay and its lack of clinical activity in patients in this study. We hypothesize that the diffusion rate of dasatinib into the tumor microenvironment may be hampered by its high protein-binding capacity causing its inactivity. Moreover, other factors contribute to the mismatch between conditions in vivo and the nonphysiological in vitro test setting. Besides an overrepresentation of Src substrates on chip, the anticipated clinical activity of dasatinib or other PKIs may be potentially overestimated by the reduced kinase specificity for the synthetic short peptides, as both the amino acid sequence context of the tyrosine phosphorylation site and the three-dimensional structure of the substrate are known to contribute to this specificity [9, 14].

It is challenging to have information on kinase or pathway activity and target specificity, affinity, and bioavailability all at one's disposal. Information of (aberrant) kinase activity in tumor cells from patients is dependent on relative abundance, energy, and phosphorylation status of the cells, which is balanced by activity of kinases and phosphatases. Information on PKI potency and selectivity for target kinases can be obtained by high-throughput screening platforms [15]. Verification of adequate PKI accumulation in tumor tissue in patients during treatment is less straightforward. Tumor concentrations are not properly reflected by circulating concentrations in blood, as we and others have previously shown [13, 16, 17]. Of interest, alternative approaches to predict tumor PKI concentrations may become available (e.g., by imaging using labelled drugs) [18, 19].

The benefits of the kinase activity-profiling microarrays used in this study over other strategies include their high-throughput usability and limited protein input requirements, enabling their implementation in clinical practice. However, label-free mass spectrometry-based tyrosine-phosphoproteomics may be a complementary approach, as this allows for more unbiased and direct inference of signaling pathway or kinase activity. We have recently shown that this approach is feasible in small clinical samples, allowing the identification of patient-specific but also PKI-specific profiles [20] (and Labots et al., unpublished data).

In conclusion, we here show that tumor needle biopsies from patients with refractory solid tumors provide sufficient tissue to reliably determine PKI inhibition profiles. However, this microarray-based PKI selection strategy was insufficient in predicting subsequent clinical benefit upon treatment with the selected PKI. We anticipate that the PKI concentrations used in this in vitro assay should be re-evaluated based on achieved PKI tumor concentrations in patients. In addition, PKI-affinity/selectivity and mass spectrometry-based (tyrosine) phosphorylation profiles may further guide development of predictive tools or biomarkers for PKI treatment benefit. Such an improved strategy is of utmost importance to realize the promise of personalized medicine for treatment selection in this high-need patient population.

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Disclosures

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Figures and Tables

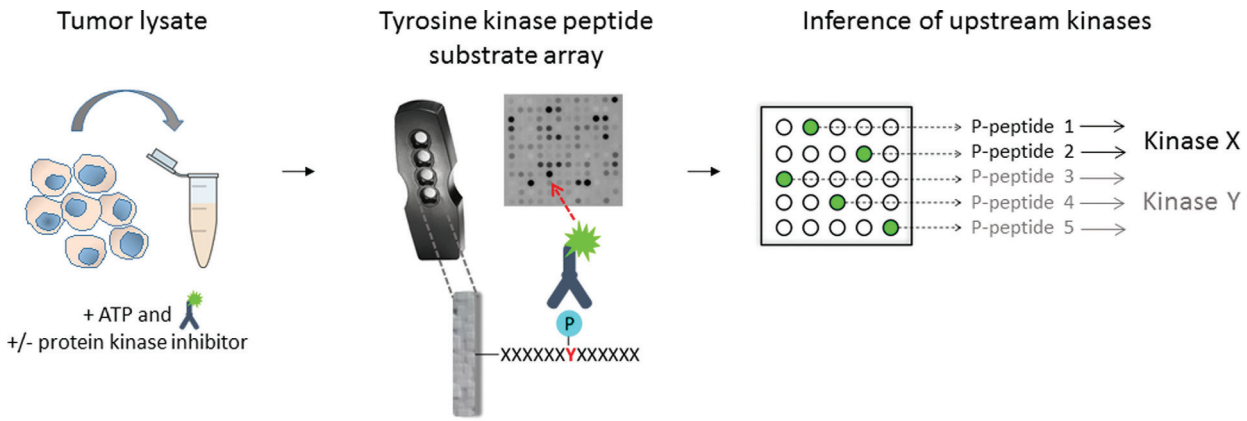

Figure 2. Kinase activity measurement based on the PamChip (tyrosine kinase peptide substrate) microarray using a PamStation12 instrument. Per patient, control and inhibition samples were measured in triplicate using 5 μg lysate protein input per sample. Each run, based on three chips with four microarrays each, allows for simultaneous measurement of 12 samples. Shortly before application on the microarray, tumor lysate is mixed with kinase reaction buffer, containing the fluorescein-labeled antiphosphotyrosine antibody pY20 as well as ATP, for phosphate transfer. In addition, for the inhibition samples, protein kinase inhibitors (PKIs) were spiked to the sample mix. Hereafter, incubation of the microarrays at 30°C is started for 60 cycles, during which the sample mix is transferred through the porous array once per minute. As a result of lysate kinase activity, (target) peptide substrates on chip will be phosphorylated at the tyrosine residue (Y), leading to phosphotyrosine formation, to which the fluorescein-labeled antibody will bind. A 12-bit charge-coupled device camera monitors fluorescence intensities resulting from binding of the antiphosphotyrosine antibody over time. End levels of signal intensity, expressed in arbitrary units, after 60 minutes of incubation were determined for PKI-spiked and control lysates. For each PKI, the percentage inhibition for all 144 peptide substrates on chip was calculated by dividing the mean end-level signal intensity of the PKI-spiked sample triplicates by the mean end-level signal intensity of the control sample triplicates (end-level intensity PKI/control). Peptide phosphorylation inhibition was considered to be significant if the *p* value calculated from a Student's *t* test was $<.05$. Kinase enzymatic activity can be inferred from recorded intensity of peptide phosphorylation over time. XXXXXXYXXXXXX denotes peptide sequence context with tyrosine (Y) substrate flanked by six other amino-acids.
Abbreviations: ATP, adenosine triphosphate; P-peptide, phosphorylated peptide.

Inhibition, %	Number of significantly inhibited peptide substrates per PKI			
	1–10	11–20	21–30	> 30
0	0	0	0	0
1–20	0	1	2	2
21–40	1	2	3	3
41–60	2	3	3	4
>60	3	4	4	4

Algorithm score	Interpretation of PKI activity	Treatment selection
0	Absent	–
1	Low	–
2–3	Intermediate	+
4–5	High	+
≥6	Very high	+

Figure 3. Per PKI, extrapolation of the ex-vivo potency to their (potential) activity in patients was based on an algorithm considering the number of significantly inhibited peptides (columns) with, in the rows, their individual average percentage of inhibition (*left*). A PKI was considered to demonstrate no (significant) phosphorylation inhibition if the sum of the scores obtained from the algorithm was 0, low inhibition if the sum of the scores was 1, intermediate if 2 or 3, high if 4 or 5, and very high inhibition if this score was $\geq$6. In vitro, a PKI should at least result in intermediate phosphorylation inhibition to be considered significant and to thus be selected for therapy (*right*). In case $\geq$2 PKIs would display intermediate to very high inhibition, the agent with the highest cumulative score was selected for treatment of the patient. In case of equal scores, the least toxic drug was selected for treatment.
Abbreviation: PKI, protein kinase inhibitor.

Table 1. Patient characteristics

Patient ID	Age, years	Gender	Diagnosis	Selected PKI	Response at week 12	Response at week 6	PFS in weeks	Grade ≥3 toxicity (possibly related)
1	63	M	Rectal cancer	Dasatinib	NE[a]	NE[a]	NE	
2	62	F	Colon cancer	Dasatinib	PD	SD	12	Deep venous thrombosis
3	68	M	Rectal cancer	Dasatinib	NA[b]	NA[b]	NA	
4	57	M	Cholangiocarcinoma	Dasatinib	NE[a]	NE[a]	NA	Pain right flank; Fatigue
5	38	M	Germ cell cancer	Erlotinib	PD	PD	6	
6	57	F	Colon cancer	Dasatinib	PD	PD	6	Fatigue
7	26	M	Synovial sarcoma	Dasatinib	PD	PD	6	
8	64	F	Cholangiocarcinoma	Dasatinib	PD	PD	6	
9	57	M	Pancreatic cancer	Sunitinib	PD	PD	6	
10	69	F	Gallbladder cancer	Sunitinib	SD	SD	20	
11	62	F	Cholangiocarcinoma	Dasatinib	PD	PD	6	
12	66	M	Colon cancer	Dasatinib	NE[c]	NE[c]	NE	

Patients for whom peptide microarray-based kinase activity profiling was performed.
[a]Patient not evaluable due to rapid deterioration.
[b]Not applicable; patient turned ineligible after profiling.
[c]Patient not evaluable, declined treatment after profiling.
Abbreviations: 6, PFS is 6 weeks; F, female; ID, identification; M, male; NA, not applicable; NE, not evaluable; PD, progressive disease; PFS, progression-free survival; PKI, protein kinase inhibitor; SD, stable disease.

Table 2. Summary algorithm scores

Patients with selected PKI, *n*	Median algorithm score (range) for PKI in the test		
	Dasatinib	Sunitinib	Erlotinib
Dasatinib, $n = 9$	**8.2 (6–10)**	5.0 (3–6)	2.5 (1–5)
Sunitinib, $n = 2$	8.5 (8–9)	**9.5 (9–10)**	8.0 (7–9)
Erlotinib, $n = 1$	6 (NA)	6 (NA)	**7** (NA)

Table displays the selection algorithm scores for the PKIs dasatinib, sunitinib, and erlotinib (columns), reflecting their in vitro inhibitory potency, in the 12 patients (rows) for whom kinase activity profiling was performed. The median selection algorithm score for dasatinib in all 12 patients was 8 (range 6–10). In the nine patients for whom dasatinib was selected, this was 8.2, whereas the median score for sunitinib was 5.0 and 2.5 for erlotinib. For the two patients with sunitinib as most active drug in vitro, differences between the top three drugs were smaller. In these patients, the median algorithm score was 9.5 for sunitinib, 8.5 for dasatinib, and 8.0 for erlotinib.
Data for lapatinib, everolimus, and sorafenib are not shown; the median algorithm selection score of these drugs in all 12 patients was 1 (range 0–6).
Abbreviations: NA, not applicable; PKI, protein kinase inhibitor.