

Kinase Inhibition with BAY 43–9006 in Renal Cell Carcinoma

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ABSTRACT

BAY 43–9006 is an oral inhibitor of CRAF, wild-type BRAF, mutant V599E BRAF, vascular endothelial growth factor receptor (VEGFR) 2, VEGFR3, mVEGFR2, FLT-3, platelet-derived growth factor receptor, p38, and c-kit among other kinases. A Phase I study of BAY 43–9006 identified 400 mg orally twice daily as the recommended Phase II dose. The Phase II results of a study of BAY 43–9006 at 400 mg orally twice daily were particularly interesting in patients with renal cell carcinoma. Data from the first 41 patients with renal cell carcinoma showed that 30% of patients had stable disease (defined as between 25% reduction and 25% growth), 40% had responded (defined as >25% reduction), and 30% had progressed. Disease could be stabilized for periods in excess of a year. Some lesions became cystic and could actually enlarge while developing a low attenuation core. This phenomenon is recognized in the treatment of gastrointestinal stromal tumors with imatinib mesylate. The toxic effects of BAY 43–9006 were manageable and included hypertension, edema, diarrhea, hand and foot syndrome, rash, and hair loss where the rash involved the scalp. There was an impression of tachyphylaxis such that patients who required a dose reduction could be restored to full dose after a few months. A Phase III randomized, placebo-controlled trial of BAY 43–9006 has started for patients whose renal cell carcinoma has progressed within 6 months of immunotherapy. Combination studies with interferon, interleukin 2, bevacizumab, and chemotherapy are under consideration. The therapeutic targets of BAY 43–9006 in renal cell carcinoma remain unclear. Unlike melanoma, BRAF mutations have not been found in renal cell carcinoma. Other candidate targets include VEGFR2 and VEGFR3.

INTRODUCTION

Intracellular signaling pathways are known to control specific cell functions such as proliferation, differentiation, and cell death (1). One example is the RAS-RAF-mitogen-activated protein (MAP)/ extracellular signal-regulated kinase (ERK) kinase (MEK)-ERK-MAP kinase pathway, which is controlled by receptor tyrosine kinase activation (Fig. 1). Activation of RAS

proteins at the cell membrane in turn leads to phosphorylation and additional downstream activation of RAF, MEK, and ERK (2), forming an intracellular cascade system and resulting in changes in transcription, metabolism, and cytoskeletal arrangements within the cell (3). This pathway is an important mediator of tumor cell proliferation and angiogenesis, and aberrant activation, for example, due to activated RAS or mutant BRAF, is known to be a significant determinant of the malignant phenotype in a variety of solid tumors (4).

Several lines of evidence implicate the RAS/RAF pathway in the pathobiology of renal cell carcinoma. A cytogenetic study on human renal cell carcinoma revealed deletions within the short arm of chromosome 3, with resultant shift of *CRAF* locus from *3p25* to *3p14*, demonstrated by *in situ* hybridization with a *CRAF-1* probe (5). In addition, activated *H-RAS* oncogenes have been detected in human renal cell carcinomas with single point mutations within codons 12 and 61, thus indicating that these genetic lesions that affect RAS may serve as one of the multisteps in the carcinogenic process (6). Finally, constitutive activation of MAP kinases, which are downstream components of this signal transduction pathway, has also been implicated in renal cell carcinogenesis (7). In this study, it was demonstrated that overexpression of MEK was significantly associated with MAP kinase activation in 25 human renal tumors. The findings from these studies suggest that inhibition of the RAS-RAF-MEK-ERK signaling pathway in renal cell carcinoma may be therapeutically relevant.

BAY 43–9006 is a novel bi-aryl urea developed by BAYER Pharmaceutical Corporation and Onyx, which was designed as a small molecule inhibitor of the RAF proteins CRAF and BRAF *in vitro* (Fig. 2). Furthermore, subsequent characterization of this new drug using *in vitro* kinase assays revealed it to be a multitargeted inhibitor with activity against several other receptor tyrosine kinases such as vascular endothelial growth factor receptor (VEGFR)2, VEGFR-3, and platelet-derived growth factor receptor B (PDGFR-B) among others (Table 1), which are also involved in neovascularization and tumor progression. Laboratory studies with BAY 43–9006 demonstrated inhibition of the MAP kinase pathway in several tumor cell lines, including colon, pancreas, and breast, expressing mutant K-RAS and wild-type and/or mutant BRAF. Broad-spectrum antitumor activity has also been shown in corresponding tumor xenograft models, with confirmation of inhibition of ERK phosphorylation by immunohistochemical analysis (8).

Phase I studies of BAY 43–9006 involving 163 patients treated with different continuous oral dosage schedules revealed high interpatient variability in pharmacokinetics with adequate tolerability at a continuous oral dose of 400 mg twice daily (9). Pharmacodynamic results confirmed inhibition of CD7-positive T-cell activation and, thus, biological activity at this dose level. Dose-limiting toxic effects included grade 3 diarrhea, hand-foot syndrome, and fatigue, all of which were reversible on discontinuation of drug use. Seven patients with renal cell carcinoma were treated, with 1 partial responder and 5 patients with stable

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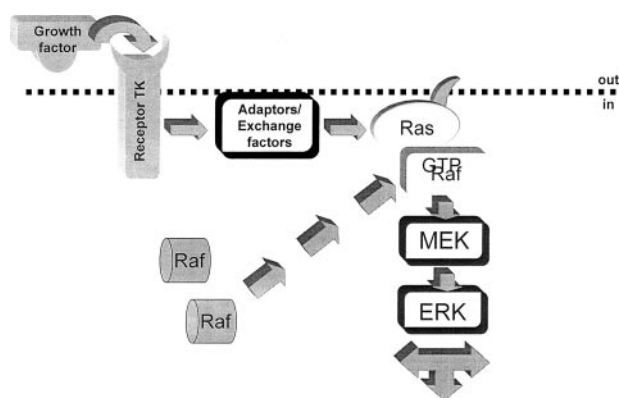


Fig. 1 The RAS/RAF/ERK signaling pathway.

disease. These data supported additional development of this kinase inhibitor in the context of Phase II clinical trials.

BAY 43-9006 has been the subject of a large, Phase II, multicenter, randomized discontinuation trial, which, although designed with power to detect significant antitumor activity in patients with advanced colorectal cancer, allowed recruitment of patients with a range of other tumor types including renal cell carcinoma. The randomized discontinuation design (10) was selected to enable evaluation of the activity of this putative cytostatic agent, because it was initially anticipated that BAY 43-9006 may slow or even arrest tumor growth resulting in disease stabilization (rather than exerting true cytostatic effect). All of the patients received single-agent BAY 43-9006, 400 mg twice daily continuously, for a 12-week run-in period. At 12 weeks, a radiologic evaluation (computed tomography and/or magnetic resonance imaging) was performed, and patients with objective responses ($\geq 25\%$ tumor shrinkage) continued with the drug indefinitely, until disease progression. Patients with stable disease after the 12-week run-in phase were randomized in a double-blind manner to either active drug or oral placebo. If after this stage any randomized patient progressed on the treatment, their randomization code was broken, and those receiving placebo were rechallenged with open-label BAY 43-9006. Patients were taken off study with discontinuation of BAY 43-9006 use in the event of disease progression while taking the active compound at any stage of the trial. Tumor evaluation occurred every 12 weeks after baseline imaging (0, 12, 24 weeks, and so on).

CLINICAL RESULTS

Recruitment to this trial was completed in January 2004, and updated results are currently awaited. However, preliminary data are particularly interesting in the cohort of patients with renal cell carcinoma (11). Analysis of patient characteristics in this group revealed that most were receiving the trial drug as second-line (56%) or third-line (34%) therapy. All of the patients were performance status 0 or 1 and 56% had undergone prior nephrectomy. The 12-week assessment in the renal cell cohort revealed that 30% had stable disease (defined as response between 25% reduction and 25% growth) and 40% had responded ($>25\%$ reduction). Progressive disease was seen in

30% of the renal patients. The rationale behind the nonstandard response definitions stems from the expectation of slow tumor responses, because it was anticipated that the drug would fulfill a role as maintenance treatment. Several points are noteworthy among these results. The responses were gradual in most cases, although rapid shrinkage in mass lesions has also been reported (Fig. 3). Response and ongoing tumor shrinkage could continue for >6 months. Some tumors, however, demonstrated cystic degeneration and enlargement on treatment with the development of a low attenuation core (Fig. 4). Interestingly, this phenomenon has been recognized in the treatment of gastrointestinal stromal tumors with imatinib mesylate, another novel signal transduction inhibitor. These observations suggest that scan results must be interpreted with caution, because apparent increase in tumor size may not necessarily reflect true disease progression.

Toxicity data from the study are encouraging with the adverse effects manageable in most patients. Notable events included hypertension that responded to standard medication (diuretics and/or β -blockade), and it has been postulated that increases in blood pressure may be associated with response. Other toxic effects included edema that occasionally necessitated dose interruption, diarrhea, and hand-foot syndrome (particularly affecting pressure points and responsive to pyridoxine and topical emollients), rash, and, in some cases where the rash involved the scalp, alopecia. Interesting observations included examples of significant hair growth, particularly in patients receiving treatment for >6 months and also a clear impression of tachyphylaxis, enabling patient treatment to be restored at full dose a few months after dose reduction due to toxic effects.

DISCUSSION

These preliminary results from the randomized discontinuation study have prompted additional interest in the clinical development of BAY 43-9006 as a novel signal transduction inhibitor. A Phase III randomized controlled trial of single-agent

- Bayer Pharmaceuticals/ONYX have produced a small molecule inhibitor of RAF proteins: BAY 43-9006
- BAY 43-9006 is an orally available potent inhibitor of CRAF: IC₅₀ ~ nM

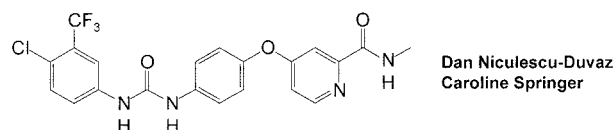


Fig. 2 BAY 43-9006: a c-RAF inhibitor.

Table 1 Targets of BAY 43-9006

Kinase assay	IC ₅₀
C-RAF, mVEGFR2	<10 nM
VEGFR3	10–20 nM
wt B-RAF, V599E B-RAF, p38, PDGFR	20–40 nM
FLT-3, c-KIT	40–80 nM
VEGFR2	80–160 nM
EGFR, PKC, MEK, ERK	Inactive at 10 μ M

Dan Niculescu-Duvaz
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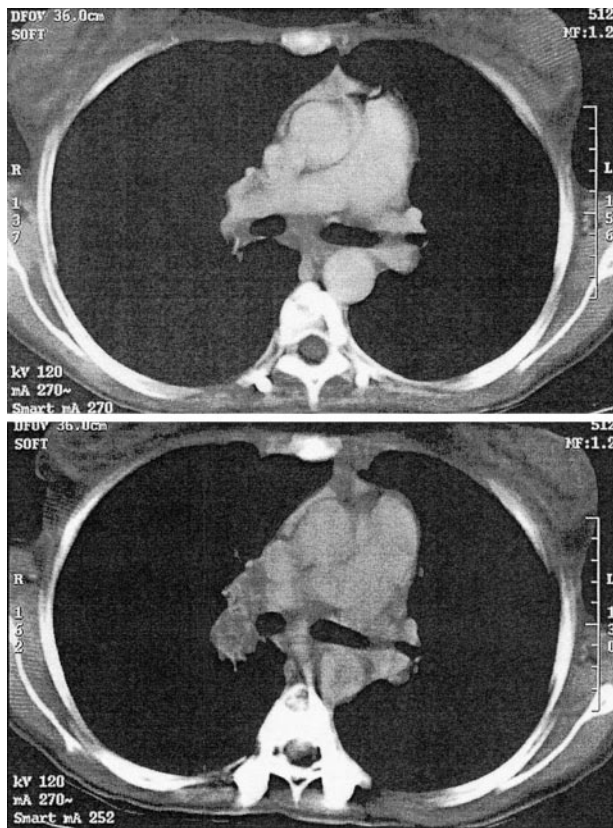


Fig. 3 Computed tomography scan showing response with shrinkage of tumor.

BAY 43-9006 (Sorafenib) *versus* placebo has commenced and is planned to accrue 884 patients with renal cell carcinoma who have progressed within 6 months of immunotherapy with interferon or interleukin 2. The study is due to complete accrual by summer 2005 and has been designed to detect a 33.3% increase in overall survival (540 events required) and a 50% increase in time to progression (303 events needed). An interim analysis is planned by the data monitoring committee after 270 events.

If current studies and the results from the trial described herein continue to support the therapeutic value of this kinase inhibitor in a relapse setting, it would be interesting to compare BAY 43-9006 against our current standard of care with immune modulators such as interferon or interleukin 2 as upfront treatment for newly diagnosed cases.

In addition to its use as a single agent, there is also interest in combining BAY 43-9006 with other drugs to maximize therapeutic potential. However, the issue of which agents are the most suitable for combination studies remains to be resolved. Possible candidates include interferon and interleukin 2, which have proven activity in renal cell carcinoma. However, biomarker data from the Phase I studies of BAY 43-9006 indicated that the drug blocked T-cell activation at the recommended dose, thus suggesting possible antagonism of these immunotherapy agents, resulting in a counterproductive combination.

Another logical choice for combination studies is bevacizumab, a monoclonal antibody directed against VEGF receptor,

which has been shown to prolong survival in a small Phase III renal cell carcinoma trial (12). Finally, the feasibility of combining BAY 43-9006 with chemotherapy has also been raised. Although renal cell carcinoma is well known to be a relatively chemoresistant tumor, parallel studies using BAY 43-9006 with carboplatin and paclitaxel (13) in malignant melanoma, another chemoresistant cancer, have demonstrated promising results, potentially paving the way for similar trials in renal patients.

In addition to the clinical program, the scientific development of BAY 43-9006 must also continue, because it has yet to be fully determined how this drug is exerting antitumor activity in renal cell carcinoma. Although the compound was initially designed as a RAF inhibitor (CRAF and BRAF), preliminary studies to date have not demonstrated BRAF mutations in renal cell carcinoma (14) unlike in malignant melanoma, which has been found to exhibit BRAF mutations in ~70% of cases (15). Alternative targets for the drug in renal cell carcinoma have been postulated, the most frequent being VEGFR2 and VEGFR3, which appear to play an important role in the angiogenesis of this disease (16) and are inhibited by BAY 43-9006. Studies with the VEGF-receptor tyrosine kinase inhibitor PK787 demonstrated antitumoral and antiangiogenic activity in murine renal cell carcinoma models (17). Preliminary data suggest that this drug can be administered safely, and the efficacy data appear promising. Additional support for VEGF pathway inhibition can be derived from a randomized Phase II trial comparing the anti-VEGF antibody bevacizumab with placebo. This study involved 116 patients with metastatic renal cell carcinoma and revealed that this drug can significantly prolong the time to progression of disease, although no significant differences in overall survival were seen between groups (18).

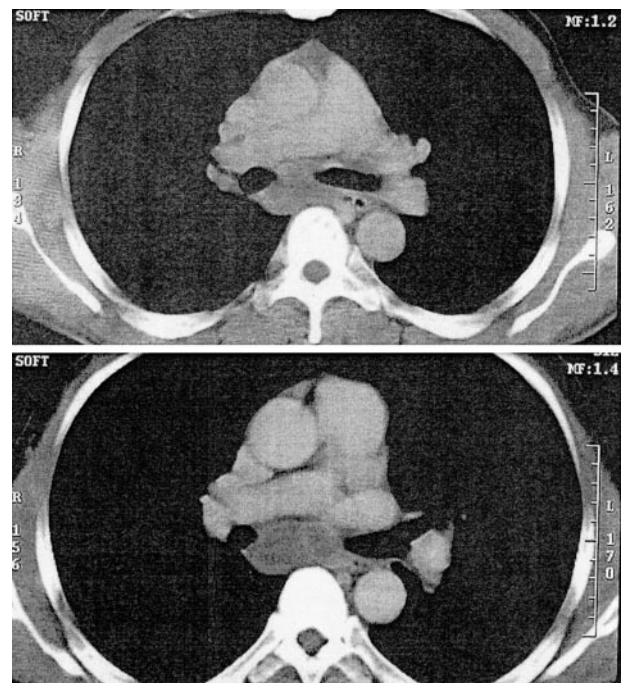


Fig. 4 Computed tomography scan depicting cystic degeneration of tumor.

Another potential mode of action of BAY 43-9006 in renal cell carcinoma may be through PDGFR, which is also inhibited by the drug (Table 1). Platelet-derived growth factors exert their biological activity by binding to three different tyrosine kinase receptor isoforms, in particular the PDGFR α , which is associated with growth stimulation (19). The expression of this receptor and its ligands was studied in human renal cell carcinoma and found to be significantly higher in grade 3 and 4 tumors, and higher receptor expression correlated with tumor progression (19).

Another possible candidate is c-kit, with a BAY 43-9006 IC_{50} of 40 to 80 nmol/L. However, in a study that analyzed gene expression profiles in 15 renal cell carcinomas (conventional, papillary, and chromophobe) using high-density oligonucleotide arrays, although the oncogene kit was up-regulated, this was found specifically in chromophobe renal tumors and not in conventional renal cell carcinoma, the dominant histologic subtype (20).

Interestingly neither protein kinase C nor epidermal growth factor receptor (EGFR) appear to be inhibited by BAY 43-9006 according to the pharmaceutical data available. However, it has been suggested in the literature that inhibitors of both these signaling system components may have a role in the treatment of renal cell carcinoma. This hypothesis has been tested with varying results. A Phase II trial of bryostatin, a protein kinase C inhibitor, involving 32 patients with advanced renal cell carcinoma produced a low proportion of objective responses (21). Nevertheless, prolonged stable disease or partial remission in 25% of patients was seen, suggesting that there may be therapeutic potential.

EGFR and its ligands epidermal growth factor and transforming growth factor α are overexpressed in human renal cell carcinoma, compared with normal renal tissue (22), and EGFR signaling mechanisms have been associated with development and progression of renal cell carcinoma metastases. Conversely, blockade of EGFR signaling decreases proliferation of renal carcinoma cells *in vitro* (23). Furthermore, it has been demonstrated that down-regulation of EGFR signaling by a novel oral EGFR tyrosine kinase inhibitor PKI 166 not only markedly inhibits cell proliferation *in vitro* but can retard the growth of human renal carcinoma xenografts implanted into nude mice (23) when the drug is used alone or in combination with Taxol. However, clinical studies have not corroborated these data. A multicenter Phase II trial of 55 patients with metastatic renal cell carcinoma treated with the anti-EGFR antibody C225 (Cetuximab) failed to demonstrate any responses (24).

The data described herein suggest that there may be other important targets to be considered, and additional research into the mutual interactions among intracellular signaling pathways is needed to help elucidate the mode of action of BAY 43-9006, other potential kinase inhibitors attractive for clinical development, the choice of agents for selection in combination studies, and possible identification of sensitivity markers, which will ultimately allow improved patient selection for treatment.

OPEN DISCUSSION

Dr. Kaelin: If I am not mistaken, SU 5416 is not a very good PDGF-inhibiting drug. It doesn't hit it at all, whereas BAY

43-9006 does. In fact, there seems to be a correlation in that BAY 43-9006 and SU 11248 might be the most active agents thus far in the clinic, and both inhibit both VEGFR and PDGFR. That doesn't explain the bevacizumab experience, however.

Dr. Tim Eisen: There is this discrepancy in the bevacizumab experience and certainly other antibody treatments. They may work by a similar pathway, but they are different drugs. I wonder, Jim, whether you have a comment about that in renal cell. What is the importance of the method of targeting a pathway?

Dr. James Yang: I think we know a lot more about the targets, but we don't know the whole availability and pharmacokinetics.

Dr. Michael Atkins: Are there any data on VEGF levels in this patient population? Is there any evidence that there is compensatory increase in VEGF as you inhibit with this agent or with any of the other agents as you make the cells hypoxic? Is there an increase in circulating VEGF levels that would be a justification for adding a VEGF binding agent, VEGF TRAP or bevacizumab, in combination with BAY 43-9006?

Dr. Eisen: I'm not aware of any such data.

Dr. Walter Stadler: I've heard some rumors to that effect, but I haven't seen the data.

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