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Tipifarnib in hematologic malignancies

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I. Rationale for using Tipifarnib in hematologic malignancies

Signal transduction pathways regulate the cell processes of growth differentiation and survival. Aberrations in signal transduction pathways can lead to increased cell proliferation, apoptosis inhibition, increased angiogenesis, tissue invasion and metastasis. Most human neoplasias have aberrant signal transduction elements. Therefore signal transduction inhibitors represent a new class of compounds potentially active in cancer treatment. These agents target proteins or pathways that are involved in the pathogenesis of malignancies.

Farnesyl transferase inhibitors (FTI) represent one class of signal transduction inhibitor that has been explored in hematological malignancies. FTI are potent and selective inhibitors of intracellular farnesyl protein transferase (FPPase). This enzyme catalyses the transfer of a farnesyl moiety to the cysteine terminal residue (sulfhydryl cysteine of the CAAX motif) of substrate proteins.^{1,2}

A number of intracellular proteins are substrate for such farnesylation and are potential target for FTI, including Ras proteins. The Ras family of genes encodes proteins that are involved in key cellular fraction such as proliferation, survival and differentiation.^{3,4} To be fully active Ras must transfer from the cytoplasm to the inner surface of cell membrane. Localization of Ras protein to the cell membrane requires prenylation (or farnesylation) mediated by FPPase.^{1,4} Mutation of Ras or other genetic abnormalities can induce constitutive activation.

Ras was considered as a relevant target for anti-cancer therapy not only because of its crucial role in signal transduction cascades but also because activation of Ras is one of the most frequent genetic aberrations in cancer.^{5,6}

Therefore, FTI were initially developed with the objective of targeting Ras and in cancers associated with a high incidence of Ras mutations. Downstream of Ras, there

are different pathways that are involved in important cellular functions. One of these pathways is the Raf-MEK-MAPK cascade in which a series of phosphorylation events leads to the localization of phosphorylated MAPK to the cell nucleus, where it activates transcription factors that control cell proliferation and apoptosis.^{7,8}

A second important Ras mediated signalling cascade is the PI3K-AKT pathway in which Ras activates PI3K, a tyrosine kinase that phosphorylates AKT which is a suppressor of apoptosis.^{9,10}

However, recent clinical data suggest that the presence of Ras mutations is not required for the antiproliferative effect of FTI.¹¹ The growth inhibition properties of FTIs may be attributed to their effects on other farnesylated proteins. These potential FTI targets include small G-proteins (like Rho proteins),^{12,13} centromere binding proteins (CENP-E, CENP-F)^{14,15}.

Tipifarnib (Zarnestra[®], R115777) is a selective and orally bioavailable FTI that inhibits a variety of human tumor cell lines *in vitro* and *in vivo*.^{16,17} Several arguments justified the evaluation of Tipifarnib in acute myeloid leukemia (AML). Observations from patient-derived samples in culture indicate that leukemic blasts are more sensitive to the cytotoxic effect of tipifarnib than are normal cells.¹⁸ AML cells may constitutively express effector or activate pathways that are targeted by FTI or and that are involved in cell proliferation and survival. A critical role of Ras in leukemogenesis has been suggested by an *in vivo* model (using irradiated mice reconstituted with bone marrow transfected with activated Ras.¹⁹ If Ras protein is considered as the main target of FTI, Ras is frequently dysregulated in AML.⁷

II. Clinical experience with Tipifarnib in acute myeloid leukemia

II.1. Phase I study of Tipifarnib in acute leukemias

The biologic and clinical effects of Tipifarnib in acute leukemia were first tested

Table 1. Response rates Phase II study of Tipifarnib.

	Relapsed	Refractory	Total N=252	% intention to treat	% per protocol N=169
CR or CRP	7	4	11	4	7
Leukemia free status (<5% BM blasts)	12	7	19	8	11
> 50% reduction in BM blasts	18	9	27	11	16

in a Phase I dose-escalation trial.¹¹ In this study Tipifarnib was administered orally in 34 evaluable adult patients with poor risk acute leukemias at doses ranging from 100mg twice daily (bid) to 1200 mg bid for up to 21 days. Their median age was 65 (range 24–77). Twenty five patients had AML (6 newly diagnosed with poor-risk characteristics, 9 relapsed, 10 refractory to induction or reinduction treatment), 6 had acute lymphoblastic leukemia (ALL) (including 3 Ph1+ ALL) and 3 had chronic myeloid leukemia (CML) in blast crisis.

Dose limiting toxicity occurred at 1200mg bid with 2 grade 3 central nervous toxicities (ataxia, confusion and dysarthria). Non-dose-limiting toxicities (grade 1/2) included fatigue (7 patients), nausea (9 patients), polydipsia and renal dysfunction (6 pts including 4 pts at the 900mg bid level). At 900 mg bid, 6/11 patients experienced one or more grade 1/2 toxicities.

Drug-induced myelosuppression was detected mostly at 600mg and 900 mg bid. The WBC nadir occurred on median day 16. A nadir of 500 WBC/ μ L was observed in 2/8 patients at 600mg bid and 5/8 patients at 900 mg bid. Clinical responses occurred in 10 patients (29%) including 2 complete remissions (CR) and 8 partial remissions (PR) (with normalization of peripheral blood counts but 5–25% blasts in the marrow). Responses were observed at all dose levels.

FTPase activity was inhibited in all patients receiving 300mg bid and higher doses. Protein farnesylation was inhibited consistently in patients receiving 600mg bid as detected by accumulation of prelaminin A and of pre-HDJ-2. After Ras activation, ERK is phosphorylated through Raf-MEK pathway. In 8/22 patients with constitutive ERK phosphorylation, phospho-ERK became undetectable after Tipifarnib treatment. The conclusions of this study were that, in advanced leukemia, Tipifarnib given orally is safe at doses up to 900mg bid and effective, and that biologic consequences of FTPase inhibition are observed with doses > 300 mg bid. An unexpected result from this trial was that N-ras mutation was not detected in any of the 34 evaluable patient samples. This finding suggests that Zarnestra could act upon signaling processes other than Ras.

II.2. Phase II study of Tipifarnib in patients with refractory or relapsed AML (INT17)

Following the encouraging results of Phase I study, a large multicentric Phase II study was completed using the regimen that maximizes drug exposure and FTPase inhibition without inducing dose-limiting toxicity (600 mg bid administered for 21 consecutive days in 4-week cycles).²⁰

The study was conducted in 13 countries and recruited 252 patients (117 relapsed, 135 refractory). However only 169 patients (66%) completed one treatment cycle (per protocol). The median treatment duration was 42 days. The median overall survival of the 11 patients who achieved CR was 369 days.

Overall, the drug was well tolerated. Myelosuppression was the major toxicity in this study. Non-hematologic toxicity was mild and grade 3–4 side effects were uncommon (25%). The incidence of febrile neutropenia was 27% and 12 deaths were related to infection. Tipifarnib was administered in the outpatient setting. Most patients (82%) were hospitalized but overall < 10% study time was spent in the hospital and 44% of patients were hospitalized for disease-related events.

In conclusion, Tipifarnib was well tolerated and showed antileukemic activity in patients with relapsed or refractory AML. However, the CR rate was < 5% which raises at least two questions.

1. Is it possible to predict which patients will respond to the drug?

A pharmacogenomic analysis was performed in parallel.²¹ Gene expression profile from 80 bone marrow samples were analyzed on the Affymetrix U133A gene chip Supervised statistical analysis identified 8 gene expression markers that could predict patient response to Tipifarnib. The most robust gene was the lymphoid blast crisis oncogene AKAP13 which was overexpressed in patients resistant to Tipifarnib.

2. Is 600 mg bid the optimal dosage?

In phase I, study responses were seen at all levels but pharmacodynamic criteria were in favour of using 600mg bid.¹¹ Actually, in a dose-ranging pharmacodynamic study of Tipifarnib, HDJ2 prenylation was stud-

ied in 23 patients before and after therapy.²² Inhibition of farnesylation was noted at all dose levels (from 100mg bid to 600mg bid) but the highest level of inhibition was noted at the 300mg bid dose. If clinical activity is related to the degree of farnesylation inhibition, dose-escalation beyond 300 mg bid might not be necessary.

II.3. Phase II study of Tipifarnib as post-consolidation therapy for AML in patients 60 years and older (INT -21)

In this open-label multicentric Phase II study patients 60 years and older with AML in first CR received Tipifarnib at a dose of 300mg bid for 21 consecutive days in 28-days cycles. Before enrollment, patients had received 1 or 2 cycles of consolidation. Results of this study are not yet available. However, based on the results of interim analysis on the first 88 recruited patients, it was considered that further accrual would not provide evidence of a benefit with Tipifarnib given as maintenance in elderly patients with AML and the study was terminated.

II.4. Phase II of Tipifarnib in previously untreated poor-risk AML

In this open-label multicentric Phase II study, elderly or poor-risk patients with untreated AML were recruited if they refused or where considered unfit for conventional chemotherapy.²³ Tipifarnib was administered orally on an out-patient setting at a dose of 600mg bid for 21 days, followed by a 1-3 week recovery period. Out of 170 enrolled patients, 148 are evaluable for response (median age 73). An unfavorable karyotype was present in 47% of patients and 79% have had antecedent MDS. The CR rate was 18% (20% in patients > 75 years). Median duration of CR was 6.4 months and median survival of CR patients was 14.4 months. The median number of days of drug received was 36 days. The incidence of grade > 3 non hematological toxicity was 43%. The hospitalization rate for Tipifarnib-related toxicity was 18% (median duration 12 days) and the toxic death rate was 5% at 6 weeks. In conclusion Tipifarnib given orally on an out-patient basis was effective and well tolerated in this cohort of poor-risk AML patients.

II: Conclusions

Tipifarnib is a novel agent that is administered orally and is well tolerated, allowing treatment initiation on an out-patient setting. It is active in poor-risk AML patients although the CR in relapsed/refractory patients is <5%. Considering these results, it is actually developed mostly as induction treatment for elderly patients unfit to undergo conventional chemotherapy. Other uses could be maintenance chemotherapy and

combination with classical cytotoxic agents in induction or consolidation chemotherapy.

However several questions remain:

- is 600 mg bid for 21 consecutive days the optimal schedule?
- are there subgroups of patients that can benefit for this agent and is it possible to predict these patients?
- what is the mode of action in AML, since responses do not appear to correlate with Ras mutations status?

III. Tipifarnib in other hematological malignancies

III.1. Chronic myeloid leukemia and chronic myeloproliferative disorders

Preclinical studies have demonstrated significant activity of FTI in CML.^{24,25} *In vitro* studies have shown that FTI can inhibit proliferation or induce apoptosis of imatinib-resistant cell lines.^{26,27} Tipifarnib has been evaluated in 22 patients with CML (including 6 CML in accelerated phase 6 in blast crisis, at a dose of 600mg bid 4 weeks every 6 weeks.²⁸ Seventeen patients (77%) were resistant or intolerant to imatinib. Seven patients (33%) achieved complete or partial hematological response and 4 of them had a minor cytogenetic response. Responses were only transient (median duration 9 weeks). Of note, responses were related to a high serum VEGF level pretreatment and a decrease in VEGF during therapy.

Based on preclinical studies, the combination of Imatinib and Tipifarnib has been evaluated in patients resistant to Imatinib.²⁹ Preliminary results of a phase I trial showed that, at the maximum tolerated dose of 400mg bid for both drugs, 6/11 patients achieved complete hematologic remission and 1 achieve complete cytogenetic remission.

In the first study, out of 8 patients with myelofibrosis, 2 had a significant decrease in splenomegaly and 2 had normalization of peripheral blood abnormalities.²⁸ In this particular trial, there was a significant toxicity and 58% of patients required dose interruption or reduction because of myelosuppression or extramedullary toxicity. However, this unusual toxicity could be explained by the 4-week schedule.

In another Phase II trial with lower doses (300 mg bid for 21 days), a clinically relevant decrease in organomegaly was observed in 11/33 patients (33%) without significant improvement, however, in anemia or bone marrow fibrosis.³⁰

III.2. Myelodysplastic syndroms

A phase I trial evaluated the 3 weeks-on/1 week-off schedule with Tipifarnib in myelodysplastic syndroms (MDS).³¹ The starting dose was 300mg bid. Dose limiting toxicities were observed at 900mg total daily dose and the maximum tolerated dose was 400 mg bid.

Objective responses were seen in 6/20 evaluable patients (30%) but only 2 of them had ras mutations.

Despite this result, the following Phase II trial used the dose of 600 mg bid which was recommended after the Phase I study in acute leukemia.³¹ This dose resulted in side effects (myelosuppression, fatigue, neurotoxicity, rash or leg pain) necessitating dose reduction or discontinuation of therapy in 41% of the 27 evaluable patients. Three patients responded, but to a reduced dose of 300 mg bid.

Another international Phase II study on 82 patients with high-risk MDS used a lower dose of Tipifarnib (300 mg bid).³³ The overall response rate was 33% (including 6 CR). Responses were durable with median duration of 14 months. Myelosuppression was the most common side effect with 24% grade 3/4 neutropenia and 37% grade 3/4 thrombocytopenia. This study confirmed that Tipifarnib is effective and has limited toxicity in MDS patients with this schedule.

Another schedule of administration was tested in a Phase I dose-escalative trial on 53 patients with MDS.³⁴ With a one week on – one week off schedule and a starting dose of 100 mg bid, the maximum tolerated dose was 1300 mg daily.

Again the most common side effect was myelosuppression. Fifteen of 51 evaluable patients (29%) responded (including 3 CR). Responses were seen in 3/6 patients treated at the lowest level and again were independent of ras mutation status.

III.3. Multiple myeloma

FTI have also been tested in multiple myeloma since in this disease Ras gene mutation occurs in 30–40% of patients. Preclinical studies have shown that Tipi-

farnib induces a dose-dependent growth inhibition and apoptosis of myeloma cell-lines and fresh myeloma plasma cells.^{35,36} Induction of apoptosis appeared to depend on the status of Ras-mutation in one study³⁷ but not in two others.^{36,38}

However, clinical experience with Tipifarnib in multiple myeloma is limited and disappointing.³⁹ At a dose of 300 mg bid for 3 weeks every 4 weeks, Tipifarnib induced only disease stabilization in 64% of patients, which was not correlated to the inhibition of HDJ-2 farnesylation and the presence of Ras mutation.

III. Conclusions

Among FTI that have been developed for cancer therapy, the largest clinical experience is with Tipifarnib. This compound has been mostly evaluated in myeloid malignancies (AML, MDS and CML). In CML, although some responses have been observed in combination with Imatinib in imatinib-resistant patients, the future of this drug appears limited with the introduction of novel tyrosine-kinase inhibitors. In MDS, preliminary results of Phase II trials appears positive and justify further evaluation, although the optimal schedule of administration is not clearly defined.

Finally in AML, the current indication of Tipifarnib given as a single agent for induction treatment of elderly patients unfit to receive conventional chemotherapy. However, the FDA recently rejected an approval application of Tipifarnib in this indication and results of the ongoing Phase III trial will be important for the future. However, given the mild non-hematologic toxicity, the future of this drug could also be the combination with cytotoxic agents in induction or maintenance therapy.

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