

PROGRESS REPORT

PLANNED TRANSLATIONAL RESEARCH PROGRAM

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Title of the project Identification of predictive factors for response to targeted therapies and biological criteria of aggressiveness in two models of connective tissue tumor with locoregional spread: aggressive fibromatosis/desmoid tumor (AF/DT) and pigmented villo-nodular synovitis/tenosynovial giant cell tumor (PVNS/TGCT)

Summary of the project:

In this study, we will investigate the molecular mechanisms of the antitumor activity of imatinib evaluated in phase II trials in two connective tissue tumors with locoregional behaviour. Specifically, we will assess kinase expression and the presence of the chimeric ligand before and after treatment in PVNS. For AF/DT, we will investigate for the presence of the M541L allele of the KIT gene, the presence of mutants of the beta catenin gene, the expression of phosphorylated forms of the target kinases of imatinib as well as other kinases.

Description of the project

1. Introduction

Several connective tissue tumors are characterized by a locally malignant locoregional behaviour, i.e. locoregional aggressiveness, but with only rare metastatic potential. This is the case for desmoid tumors, tenosynovial giant cell tumor, giant cell tumor of the bone, dermatofibrosarcoma protuberans (DFSP), low grade well differentiated liposarcomas, e.g. retroperitoneal sarcomas

Complete surgical removal remains the optimal treatment but may be difficult or mutilating according to the tumor location or local extension. Radiotherapy is discussed in case of unresectable disease or limit surgical margins. Systemic therapies can provide response in case of advanced or recurrent disease but their efficacy is limited. Efficient systemic therapies would be useful in lesions not amenable to curative surgery or for which resection would be mutilating.

2. Imatinib mesylate (Gleevec®) is a specific tyrosine-kinase inhibitor whose identified targets are kit, bcr-abl, platelet-derived growth factor receptor (PDGFR) and more recently, macrophage colony-stimulating factor receptor (M-CSF R). Imatinib is the standard first line systemic treatment for CML, advanced GIST, non operable DFSP. In addition, response of AF/DT and PVNS/TGCT to imatinib have been recently reported, with prolonged PFS for some patients. The mechanisms of this anti-tumor activity remain unclear in aggressive fibromatosis, and is supposed to involve an inhibition of the M-CSF paracrine loop in PVNS, although this has not been fully documented.

3. Pigmented villonodular synovitis (PVNS), also known as tenosynovial giant cell tumor (TGCT), is a rare pathological entity affecting the synovium in young adults. Initially considered as an inflammatory reactive process, recent observations have shown that this disease may actually be a benign neoplastic process with specific genetic alterations. Indeed, a specific t (1;2) translocation, involving the collagen 6A3 gene (on 2q35) and the M-CSF (a.k.a CSF1) gene (on 1p13), is present in a fraction of tumor cells in PVNS/TGCT [1,2]. This fusion gene expressed by a fraction of the cells encodes for a fusion protein which attracts non neoplastic cells expressing M-CSFR (macrophage and monocytes), through a paracrine - "landscape"- effect [3].

Blay et al. recently reported the case of a patient with recurrent and symptomatic PVNS/TGCT following surgery, in whom surgical re-excision would have had important functional consequences. She was treated with imatinib, providing rapid tumor response ; she relapsed at discontinuation of imatinib, and a secondary response was obtained at imatinib reintroduction [4].

Although a potential contribution of the blockade of other tyrosine kinases by imatinib can not be ruled out, the frequency at which the col6A3/CSF1 fusion gene is observed in PVNS/TGCT as compared to other pathological synovial process strongly suggest that imatinib activity involves M-CSFR blockade in this disease, despite recent observation showing limited biological activity of the product of the fusion gene [5].

A phase II non randomized multicenter trial of imatinib 400mg/d in patients with non resectable PVNS is planned for activated in 2009 and should involve 20 patients to determine the efficacy (as measured by objective tumor response) of imatinib mesylate given in patients with progressive or relapsing PVNS/TGCT that can not be treated by surgery. One of secondary objectives of the study is to explore biological mechanisms of efficacy of imatinib in PVNS/TGCT.

The protocol has been writed but actually, Novartis should promote a little different phase II trial evaluating nilotinib, another tyrosine kinase inhibitor, in non resectable PVNS. Biological analysis should also be planned.

To this aim, we intent to investigate the correlation between objective clinical response observed in the trial and:

- 1) the presence of COL6A3/CSF1 fusion gene
- 2) the presence of a phosphorylated c-fms (as well as PDGFR and KIT) by Western Blot, before and after the treatment
- 3) the expression of c-fms (along with PDGFR and KIT) by IHC before and after imatinib treatment.

4. Agressive fibromatosis (AF) also known as desmoid tumors (DT) are rare fibroproliferative neoplasm, with locoregional spreading, frequently associated with either mutations in beta catenin, or within APC for AF/DT associated with Gardner's syndrome. The first standard treatment is surgical removal of the tumor, although some researchers have recently advocated the possibility of a watch and wait policy. However, when the tumor is progressing, surgery is the standard of care; if a complete surgical removal of the tumor is not feasible, radiotherapy may be proposed. After failure of locoregional treatment, or if these are not feasible, systemic therapies such as anti estrogen, nonsteroidal inflammatory drugs and chemotherapy can induce some responses in case of recurrent or unresectable disease.

Recent publications reported several cases of AF/DT responding to imatinib. On the whole, 3 publications reported 22 cases of patients treated with imatinib, leading to 6 partial response

and 5 stable disease [6-8]. The biological mechanisms underlying the cytostatic effect of imatinib in AF remain unclear.

We reported in ASCO 2007 a study of predictive factors for response to imatinib in AF/DT on samples collected in the phase II study of the French Sarcoma Group [9, 10]. The presence of M541L KIT variant, the expression of imatinib targets and cell cycle proteins on tissue microarrays, as well as classical clinical and biological factors were investigated. Forty patients, 12 (30%) males, 28 (70%) females, with a median age of 40 were included in the study between Aug 2004 and Nov 2005. Imatinib was given during one year at a dose of 400mg/d. With a median follow-up of 21 months, progression free survival (PFS) at 1 and 2 years are 67% and 53% respectively with 4 (10%) patients in partial response and 1 (2.5%) in complete response.

The biological mechanisms underlying the response of AF/DT to imatinib are unclear. The expression of KIT which represented initially the biological rationale, was later found to be mainly due to antigen retrieval procedures. Heinrich et al failed to identify predictive factors for response to imatinib in their initial series [7]. Goncalves et al. reported on a patient with AF/DT responding to imatinib and presenting with a germline variant of exon 10 of the *KIT* gene encoding for a KIT protein highly sensitive to imatinib [8]. Seinfeld et al reported the same KIT variant in three of four aggressive fibromatosis [11]. Tamborini et al later reported that exon 10 M541L substitution did not result in KIT activation [12]. Whether the presence of M541L polymorphism in AF/DT tumors plays a role in imatinib response in this tumor remains therefore unknown.

We already investigated the biological parameters correlated with objective clinical response to imatinib in AF/DT

Paraffin-embedded tumor material was collected for 34 of the 40 (85%) patients included in the study, but DNA of proper quality could be extracted in only 11 (32%) of these 34 patients, mainly because of improper fixation medium. DNA was extracted from the paraffin-embedded tissues and polymerase chain reaction was used to amplify exon 10 of *KIT*. The purified PCR product was then subjected to automated sequencing using DNA analyzer (Applied Biosystem™). Immunohistochemical analysis of the expression of KIT (Dako) PDGFRA (R&D) and B (Santa Cruz), M-CSFR (Cell Signalling), p42 ERK (Cell Signalling), phospho-Ser 473-Akt (Cell Signalling), phospho MEK 1-2 (Cell Signalling), and Cyclin D1 (Neomarkers), β catenin (Dako), E-Cadherin (Zymed) was performed on tissue microarray in all patients in whom sufficient material was available (30 of 34).

PDGFRB and β catenin expression were observed in all samples, cyclin D1 in 5 (17%) of the samples, phospho ERK in 17 (58%) of the samples, with no correlation with PFS. Of note, none of the patient with detectable cyclin D1 expression on IHC has progressed at 1 year. No expression of M-CSFR, PDGFRA, E-Cadherin, phosphoMEK1-2, or phospho Akt on ser 473 was observed in this series. Among the eleven patients in whom DNA could be extracted, one complete response and one partial response were observed (response rate 2/11, 18%), with eight (77%) stable disease, and one progressive disease. Three (27%) tumors were found to harbor the KIT M541L variant: one was the patient in complete response, two were in stable disease. One partial response and 6 stable disease were observed in 7 of the 8 remaining patients. PFS was not significantly different in patients with and without M541L variant, nor with patients without available DNA for sequencing.

None of these results can be statistically correlated to clinical response to treatment, possibly because of the limited power of the study. An extension of this analysis on a broader group of patients may change this conclusion.

To this aim, we intent to:

1) Investigate the frequency of the M541L variant using DNA sequencing in frozen tumor tissue of 192 cases of AF/DT, collected with the collaboration of a dedicated patient

advocacy group and its correlation to tumoral evolution, patients survival and response to treatment (clinical data base and tissue collection already established). The presence of mutation of beta catenin will also be investigated for possible correlation with response to imatinib.

KIT M541L variant and mutation of beta catenin were studied in 167 patients and correlated to clinical evolution of the disease. Their presence was correlated to several factors: patient gender, hormonal dependency of the tumor, Gardner syndrome, APC mutation, location of the tumor, tumor size, response to treatment, relapse.

KIT M541L was detected in 5.8% of the studied population. None but hormonal dependency of desmoid tumor was significantly associated with the presence of M541L. Find enclosed the details of these results submitted for publication.

Mutation of beta catenin was detected in 84% of cases. Several kinds of mutation were studied: T41A, S45F, S45P and deletion. No clinical factors were found to be statistically correlated with the presence of a mutation [13].

The writing of an article to synthesize all these results is ongoing.

The analysis of clinical data of 590 patients with desmoids tumor included in database is also ongoing.

2) Perform a transfection of 3T3 cell lines with an KIT M541L variant to investigate the functional consequences of the variant allele of cell growth and response to imatinib. We will use for this purpose the in vitro model recently published by our group.

3T3 cell lines have been transfected with the KIT M541L variant in the laboratory. In September 2009, we will study cell growth (spontaneously and under hormonal stimulation cf §1) and response to imatinib and hormonal therapy which seems very important too.

3) Investigate further the phosphokinome of 13 tumor samples collected and stored frozen before and after the initiation of imatinib treatment using the Kinexus technology (from Kinexus Bioinformatics Corporation in Vancouver, Canada) which assesses 652 proteins and phosphoproteins on Kinex Antibody Microarray.

Proteins have been extracted from the 13 desmoid tumors frozen sample and will be tested on Kinex Antibody Microarray next year.

4) The results obtained will then be validated on the follow-up Desminib2 trial which will compare imatinib vs NSAIDS on non resectable DT.

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